ACKNOWLEDGMENT

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FIRST INTERNATIONAL CONFERENCE ON LIVE POLIOVIRUS VACCINES

(Washington, D. C., 22-26 June 1959).

Sponsored by the Pan American Health Organization and the World Health Organization, with the cooperation of the Sister Elizabeth Kenny Foundation

Papers Presented and Discussions Held





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Editorial Note

The present volume contains all the papers delivered by the participants at the Conference, arranged in the order of their presentation, by session The transcripts of the discussions on each paper or group of papers have been edited for the sake of brevity and consistency of style All statements related to the opening or closing of sessions, and other non substantive remarks, have been deleted in order to condense the presentation as far as possible

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713 pages
with illustrations

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Preface

The following pages present the ideas and experiences of a distinguished group of experts in various fields related to the use of attenuated viruses in vaccination against poliomyelitis. This information represents the existing scientific knowledge, as of June 1959, on the vaccine strains being used in programs throughout the world.

The Conference, as well as the compilation of its proceedings in the present volume, was made possible through the cooperation of the Sister Elizabeth Kenny Foundation of Minneapolis, Minnesota. The Pan American Health Organization and the World Health Organization express their appreciation to the Kenny Foundation, and also wish to take this opportunity to thank Georgetown University for making available the excellent facilities of its Edmund A. Walsh School of Foreign Service, where the Conference was held.

The accumulation of data on the use of attenuated live polioviruses as immunizing agents is progressing at such a speed that the need for a second conference for further evaluation is already apparent. Our Organizations, again with the assistance of the Kenny Foundation, have therefore agreed to sponsor the Second International Conference on Live Poliovirus Vaccines in Washington, D.C., 6-10 June 1960. In the forthcoming Conference we hope that the remaining problems of a biological and epidemiological nature connected with this vaccine will be thoroughly analyzed, thus paving the way for its larger use throughout the world.

Abraham Horwitz
Director
Pan American Sanitary Bureau
Regional Office, World Health Organization

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Chairman:
DR. CHARLES ARMSTRONG

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Dr Myron E WEGMAN, Secretary General, PASB

FIRST SESSION

MONDAY, 22 JUNE 1959

Chairman

DR GAYLORD W ANDERSON
Director, School of Public Health
University of Minnesota
Minneapolis, Minnesota

INTRODUCTORY REMARKS

Dr Abraham Horwitz, Director
Pan American Sanitary Bureau
Regional Office, World Health Organization
Washington D C

Dr. Hugh H Hussey, Dean
School of Medicine
Georgetown University
Washington, D C

Dr. A M-M Payne
Chief Medical Officer
Endemo-Epidemic Diseases,
World Health Organization
Geneva, Switzerland

TOPIC I. GENERAL CONSIDERATIONS

Presentation of Paper by.

Prof. George W A Dick

TOPIC II. CRITERIA OF ATTENUATION, DEVELOPMENT, SELECTION, AND TESTING OF POLIOVIRUS STRAINS FOR USE IN FIELD TRIALS

Presentation of Papers by:

Dr Albert B. Sabin

(DISCUSSION)

Dr. Roderick Murray and
Dr Ruth L Kirschstein

Dr Joseph L. Melnick

Dr Victor J. Cabasso

Dr Hilary Koprowski

Dr M. P Chumakov

(DISCUSSION)

INTRODUCTORY REMARKS

Dr. Abraham Horwitz *(Director, Pan American Sanitary Bureau)* In the evolution of ideas in search for those truths which bear on the lives of many people it is indispensable, periodically, to pause and to analyze what is known, what still remains to be learned, and to determine the course which must be followed to reach the original objectives It is inherent in human nature, when it is foreseen that an idea is one of outstanding importance, to idealize its effects, even though the evidence at hand may not be conclusive and not all the foundations of the scientific method are met.

Progress in science has seldom been based on complete knowledge or absolute truths For certain phenomena, a few fundamental facts have permitted us to apply the partial knowledge to the benefit of human life

The possibility of inducing resistance against certain microorganisms by introducing live attenuated strains into a living body has been amply demonstrated In each specific instance there were always the problems related to the antigenic capacity, the virulence of the strains their ability to spread in nature, and the possibility that the attenuated strains might revert to their original activity But once it was possible to produce stable immunizing microorganisms, only their large-scale application in communities supplied those answers which permitted the generalization of the procedure

The above applies to what has been accomplished thus far in the use of live attenuated viruses to prevent poliomyelitis There are considerable experimental data regarding the safety and potency of virus strains There are also impressive field trials in different environments which appear to confirm the safety and potency characteristics of the viruses used However, analysis of these observations is necessary because they were obtained by different research workers and because the studies were carried on in different environments and under conditions not quite comparable The analysis is also justified because the trials have brought to light additional questions meriting careful study, even though these questions may have no bearing on the essential facts which support the application of the vaccine to humans For example, it is necessary to discuss the methods used to differentiate the vaccine viruses from wild viruses which cause poliomyelitis in man, the phenomena of interference during the period of reproduction in the intestines by other viruses in the same environment, the stability of the vaccine viruses when naturally transmitted, the duration of the immunity they produce—to mention only some of the questions It is even in order to philosophize on the ecological problem posed by replacing in nature active strains of a virus by others, attenuated and equally immunizing

It is one of the functions of the Pan American Health Organization and the World Health Organization to promote and coordinate research related to the major diseases of mankind Coordination involves the opportunity to interchange ideas and experiences, the possibilities of identification of thoughts and feelings toward a common purpose, also, coordination means the expression of basic sentiments of human nature toward mankind As an expression of this responsibility, and measuring the importance of this problem and the interest it has awakened, the Pan American Health Organization and the World Health Organization with the financial assistance of Sister Elizabeth Kenny Foundation have invited leading scientists of the world in this field to analyze the present status of immunization against poliomyelitis with the attenuated virus vaccine

For the proper atmosphere *for the discussions* we are fortunate to have the excellent facilities of the Edmund A Walsh School of Foreign Service of Georgetown University *It is the function* of a true university to examine everything that affects man in society It is also the procedure of a university—*according to the Greek tradition* —"to dialogue" for the analysis of any question, particularly when it is one of vital importance to the life of man It is my *belief—also my* hope

—that in such an atmosphere as is found in the true university, reason always governs emotion and objective analysis always wins over passion. I am convinced, upon glancing through the roster of the distinguished scientists gathered here today, that this will be the spirit that will prevail throughout the Conference

I wish to express, in the name of Dr Candau and my own, the heartfelt appreciation of the World Health Organization and the Pan American Health Organization to the Sister Elizabeth Kenny Foundation for their generous financial assistance, which has made this Conference possible And to Georgetown University, we extend our sincere gratitude for making these premises available I would also like to thank the scientists present today for coming to this meeting, many from long distances and at considerable personal inconvenience I do hope that the successive sessions will prove that we have a common purpose, that we agree in the major points related to that purpose, and that the differences of opinions will be discussed always bearing in mind the human beings that we serve

DR. HUGH H. HUSSEY *(Dean, School of Medicine, Georgetown University)* Dr. Horwitz, ladies and gentlemen, my statement will be brief, so that you may move quickly to the business of the day

As Dean of the Georgetown University School of Medicine, it is my pleasant duty to bid you welcome on behalf of the President and the Directors of the University, and to bring you greetings, especially from the faculty of the School of Medicine.

While you are here in Washington, if there is anything that we, at the School of Medicine, can do to assist you to make your stay pleasant, please do not hesitate to call the office of the Dean.

Georgetown is honored to have you here, and I give you the best wishes for a successful meeting

DR. HORWITZ: I will turn the chair over to Doctor Anderson, who will preside at our meetings today.

CHAIRMAN ANDERSON: Thank you very much, Dr. Horwitz.

Dr. Payne, of the World Health Organization, has the floor.

DR A M.-M. PAYNE *(Chief Medical Officer, Endemo-Epidemic Diseases, World Health Organization)*: Thank you, Mr. Chairman

Ladies and gentlemen, this Conference has been organized in some considerable haste, because the World Health Organization and the Pan American Health Organization were under considerable pressure to advise governments on the status of live polio vaccines. This has meant a great deal of very hard work, particularly for the Pan American Health Organization, and has also meant a great deal of inconvenience to you, the participants, particularly to those preparing papers Most of these were prepared at the last minute, and for that reason they could not be distributed in advance and, indeed, some of the papers we have not yet seen

This has led to considerable difficulty in the preparation of the program In many instances we had only the title of the paper and not its contents, and in most cases we had no idea of the length, so it may be that during the course of the Conference we may have to rearrange the program considerably However, it is really quite clear that the program is extremely long, and it is going to be difficult to fit it into five days

There is danger of repetition since we are all talking about the same subject, or closely related subjects We would therefore ask that you be as brief as you can, as far as compatible with the presentation of your data, and also to avoid as much as possible repetition of data or opinions which have already been stated

We have arranged for five chairmen who will rotate, changing each day. We will also appoint a small drafting group which will prepare a report of the Conference for discussion at the last session, Friday afternoon. This, however, will be a précis, and not necessarily conclusions or recommendations

The drafting group will try to give the appropriate emphasis to any divergent views which may be expressed.

The full proceedings of the Conference, including the discussions, will eventually be published

Today's program starts with a general paper which has been submitted by Professor Dick.

This paper was deliberately chosen as the first one because he poses the questions which appeared to him to be most urgently in need of solution Perhaps the solution of some or many of these will become apparent during the Conference. It is therefore proposed not to discuss this paper this morning but to come back to it on Friday afternoon when we will have seen how many of Professor Dick's questions have been answered

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cine virus was indeed different, we would not worry about its spread, and I personally would be quite happy about the use of the current attenuated poliovirus vaccines anywhere But as we cannot prove the first alternative, we must therefore consider the possibility that the second may be true, that is excreted and passaged vaccine virus is a true reversion to a naturally occurring type of virus Up till now I do not think that I have said anything with which anyone would disagree. It is quite useless to say that you believe that the excreted virus has not reverted to the naturally occurring avirulent form, unless you can produce evidence to prove the point

If excreted vaccine virus = avirulent wild virus, then either (a) it is a stable avirulent mutant, or (b) it is relatively stable

We cannot prove which of these is true It may be that the treatment which these polioviruses have had has cleansed them of all their potential for indulging in neurotropic activities and that they will remain as stable as the coelacanth It may be that they will replace all deviationist particles throughout the world and we shall all be infected with a common but stable and harmless virus which will occupy and pass peacefully from alimentary tract to alimentary tract, for evermore, under the auspices of WHO

On the other hand (b) may be true, that is, that excreted virus may be only relatively stable and in certain circumstances revert to an epidemic form We are ignorant of the conditions under which this change may occur, for the relationship between avirulent and epidemic forms of poliovirus in nature is unknown Let me give you an example. Between 1955 and 1957 there were no paralytic cases due to Type 3 virus in Northern Ireland, but we know from antibody surveys that Type 3 virus was immunizing a high proportion of small children during that period Then, in 1958, we had a localized epidemic of 20 paralytic cases due to Type 3 virus Can you tell me how this arose? Was a virulent Type 3 strain imported or was this epidemic due to a change in a previously harmless strain which must have immunized thousands of children without mishap? Have we any proof that a relatively stable avirulent vaccine virus might not behave in the same way? Can we prove that excreted vaccine viruses which might spread around a community immunizing silently for some time could not show a similar reversion to an epidemic type of virus? Incidentally, if we had fed Type 3 vaccine in the spring of 1958 in a certain area it would have been difficult to convince ourselves and others that we had not been responsible for paralyzing these children

Now, although we do not know the exact nature of the excreted virus, it has been suggested that vaccine viruses might replace and exclude more virulent strains and that in any event these vaccine viruses must certainly do less harm to the community than wild viruses This may be so, but why do we want to do any harm? Even one slip up could have a serious effect on other immunization procedures in many countries

It must be borne in mind that there are no signs that a stable situation has arisen in countries where immunizing strains have been prevalent for years such as in the example from Northern Ireland which I have just given

If the excreted vaccine virus is only relatively stable, then what environmental or host factors can change it to an epidemic strain? It is known that certain simple changes in the physical environment of tissue cultures can lead to profound changes in virus virulence, e g Dr André Lwoff wrote me that when he adapted Dr Sabin's optimum, most highly attenuated LSc 2ab strain to grow at 41°C it was highly virulent by intracerebral inoculation Paralysis and prostration were produced with four infectious particles of this highly attenuated strain growth at 41°C, whereas ten million doses of the original strain did not produce any symptoms in monkeys We do not know what ecological conditions might produce, perhaps, less dramatic changes in the virus multiplying in the gut of some individuals I appreciate that in general a change in virulence may be expected only if it is associated with a survival advantage, but at the same time what might be called accidental changes in virulence can occur, and there are probably many ecological factors which determine changes in virulence about which we know nothing

Let me give another, quite simple example of the type of problem to which we have as yet no answer. During studies of polioviruses from paralyzed children and their asymptomatic contacts, we found in one instance that the paralyzed child at a nursery school was excreting virulent virus while his healthy contact was excreting virus in the attenuated range The results of the neurovirulence tests on the virus excreted by

TOPIC I. GENERAL CONSIDERATIONS

THE EVALUATION OF LIVE POLIOVIRUS VACCINES

PROF. G. W A. DICK AND DR. D. S. DANE
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PROF DICK *(presenting the paper)* During the next few days we will be presented with a large amount of data on immunization with attenuated poliovirus vaccines, to which Dr Sabin, Dr Cox, and Dr Koprowski have devoted so much effort. We will have discussions on the properties of the various strains of virus, and will be given extensive information on antibody levels None of these data, however, must be allowed to mask the real problem associated with the use of the currently available attenuated poliovirus vaccines which has deterred many of us from plunging headlong into the live poliovirus stream This problem is stated quite simply in the words of Albert Sabin as follows "Spread of the vaccine viruses to others is an accepted fact in the immunization with live poliovirus vaccines "[1] Everyone now agrees that spread occurs with the live vaccines which have been developed so far. Any difference of opinion which the experts may have becomes insignificant in the light of this main problem, and I hope that each of them will make a real contribution to our understanding of the significance of spread and not attempt to brush aside this most important and interesting issue

Spread is an accepted fact in the immunization with live poliovirus vaccines only because it can not yet be prevented with vaccines which will immunize Until the advent of live poliovirus vaccines, no vaccines which spread have been used for human immunization since variolation was introduced into England by Lady Mary Montagu in 1721. This was advocated by several enthusiasts for over a hundred years, till forbidden by Act of Parliament in 1840, in favor of vaccination with noncontagious agents. We must appreciate right away that "variolation" with attenuated poliovirus vaccines is basically different from vaccination against smallpox or yellow fever Although yellow fever and vaccinia vaccines contain live viruses, neither of them spreads regularly to the contacts of vaccinated individuals. How happy would any of us be with vaccinia if it spread readily from person to person and if we could only test it in countries where smallpox was endemic? How many of us would be prepared to accept the widespread use of 17D vaccine if it were regularly transmitted by mosquitoes?

At the time the recent large scale trials were initiated the viruses excreted by vaccinated individuals and which spread uncontrollably could not, as far as I know, be differentiated with the available techniques from some wild strains of poliovirus At least this appeared to be the position The greatest consistency in comparative experimental results may be expected in the work of a single investigator and if we look at some of Dr Sabin's results [2] we will see that Dr Sabin himself was unable to differentiate his own Type 3 virus after human passage from natural strains This is the second point on which all of us, I believe, are agreed Let me restate it—excreted vaccine virus cannot always be differentiated from avirulent wild viruses

The excreted vaccine virus *may* be different from naturally occurring avirulent strains but unfortunately we have no way of showing this We have then these two alternatives: (1) excreted virus is different from avirulent wild virus, or (2) excreted virus is the same as avirulent wild virus

If we could prove that in spite of its resemblance to avirulent wild viruses the excreted vac

present which at the moment we do not have the technical knowledge to detect or do not suspect will be present, and secondly, there may be difficulty in detecting small amounts of the viruses we already know about. With reference to my first point I am reminded of the fact that HeLa cells had been used for years before it was discovered that many lines were contaminated with PPLO. The detection of very small amounts of known viruses which might contaminate large vaccine lots is perhaps a problem for the statistically minded, but I am sure we should not forget about it. For reasons like these there is no saying what might be fed along with attenuated poliovirus vaccines. I am raising this bogey about contaminating agents because I consider we should have some agreed standards for testing attenuated poliovirus vaccines, and also that we should bear in mind the possibility of pathogenic contaminating viruses when conducting field trials.

TRIALS

There are three things which we must try to find out from trials: (1) the safety to the individual; (2) the safety to the community, and (3) the effectiveness in protecting against paralytic poliomyelitis.

In most trials it is a good thing to have a referee who will score up the points. I need not remind you that the evaluation of the Salk trial was done by Francis, and the trial of the British vaccine was refereed by the Medical Research Council. As far as I know, most trials of attenuated poliovirus vaccines have been evaluated by individuals who have been protagonists of live virus vaccines. I know personally, from our own evaluation of certain attenuated poliovirus vaccines, how difficult it would have been to incriminate a product we believed to be safe and had persuaded our friends to take. I am sure *others have had the same feelings. Quotations* are popular in some scientific quarters and everyone engaged in the study of attenuated virus vaccines should remember what Pepys said to his brother-in-law in his hour of trial when collecting evidence for his defense "Pray learn from me this one lesson to be most slow to believe what we most wish to be true."

I need only recall some of the studies with French neurotropic yellow fever vaccines. The most devious explanations were advanced to explain the incidence of encephalitis in individuals vaccinated with certain batches. Everything but the virus of the vaccine was at times blamed.

Let us look at some of the evidence which indicates that the current attenuated strains are safe to the vaccinated individuals. In all the reports of live virus vaccine trials which I have seen it has been stated that "there has been no illness attributable to the use of the vaccines" or that "No sickness was reported following the administration of the virus,"[8] or there is some such statement as "no side reactions or accidents attributable to the use of the vaccine have occurred in vaccinees or their contacts."[9] How well can such statements be documented? In all the large-scale trials which have been done there must have been a number of deaths first from natural causes.* I hope Dr. Hale and others who have done large scale trials will discuss the surveillance of their studies in detail.

Before we can accept statements of safety from these large human experiments, we must know whether the vaccinated individuals were observed with the same care as in the Minnesota trials,[1] the trials which Sabin has personally conducted,[11] and in our own studies with SM and TN virus vaccines,[3, 4] and those of Dr Horstmann and her associates.[6] The enormous difficulties of arranging the necessary surveillance in certain countries where large scale trials are being done must be weighing heavily on those undertaking them.

Can we have the assurance of all investigators that, without doubt, not a single case of paralytic disease or aseptic meningitis has occurred which could be associated with the feeding of any of the current vaccines? If cases did occur, can we be told in detail how they were sorted out from naturally occurring infections?

If safety to the individual were our only concern it would at least be easy to measure the

* Of the 211,596 people who were fed in the ...

the paralyzed child and an asymptomatic contact are shown in *Table* 1.

In Table 1, P B is the kind of naturally occuring avirulent virus which I cannot distinguish from some excreted vaccine viruses D D is typical, in its monkey virulence, of the kind of virus we isolate from paralytic cases

TABLE 1 COMPARISON OF THE INTRACEREBRAL MONKEY NEUROTROPISM OF VIRUSES EXCRETED BY A PARALYZED CHILD AND AN ASYMPTOMATIC CONTACT

INITIALS OF CHILDREN	TYPE OF INFECTION	MONKEYS PARALYZED WHEN INOCULATED WITH THE FOLLOWING TCD_{50} OF VIRUS				
		10^6	10^5	10^4	10^3	10^2
D D	Paralytic	*3/3	3/3	3/3	1/3	0/3
P B	Asymptomatic	1/3	1/3	0/3	0/3	—

The techniques used in these tests are fully described elsewhere[8]

* Denominator: number of monkeys inoculated
Numerator number of monkeys paralyzed
— not tested

One explanation of these results was that the relatively avirulent virus of subject P.B infected subject D D. and for unknown reasons underwent a change to a more virulent form and produced a paralytic infection

Let me *briefly recapitulate what I think we are* all agreed about, and some of the unknowns

Agreed Excreted vaccine virus spreads
Agreed: Excreted vaccine virus cannot be differentiated from some wild viruses
Unknown: Excreted virus $\neq$ natural avirulent virus or
Excreted virus $=$ natural avirulent virus
Unknown: Natural avirulent virus $\rightarrow$ stable on passage or
Natural avirulent strains $\nrightarrow$ relatively stable on passage
Unknown: Relationship between avirulent and virulent virus in nature

In spite of these unknowns the current vaccines are worthy of trial, and it is comparatively easy to devise a trial to test their safety in vaccinees. If we obtain evidence of safety in vaccinees, we cannot, however, presume safety for their contacts and their contacts' contacts Let me explain. As you appreciate, vaccine viruses are given in very large doses and are highly attenuated. *The contacts of vaccinated individuals,* however, must receive a very much smaller dose of a less attenuated virus. The vaccine virus may take an appreciable time to change when growing in the vaccinee's gut, as we found with Koprowski's TN Type 2 virus[3] This fact may make it safer to receive vaccine out of the bottle than casually from your friends' guts.

I would like to draw your attention to the fact that the degree of attenuation of the virus fed is not necessarily reflected in the degree of the *attenuation found in the excreted virus I will* give you two examples Allowing for imperfections in my own techniques, I can see little difference in the neurotropism of the virus excreted by Sabin's child subjects A S and D S fed his optimum attenuated strain,[11] and the virus excreted by two of my child subjects C D and J M D. fed with Koprowski's Type 1 SM vaccine in the bad old days[4] My second example is from children fed with the highly modified TN Type 2 virus which had a clear-cut marker characteristic of being non-cytopathogenic. The virus excreted by some children fed with this virus had the same range of neurotropism in monkeys as one would have expected if the children had been fed with a Type 2 virus from a paralytic case, and in addition, had become cytopathogenic.[3]

When I am on the subject of vaccine safety I would like to mention the problem of viruses which may contaminate the tissue cultures used in vaccine production What we have to consider is first, that there may be infectious agents

naturally occurring strains produce a long lasting immunity. The hope of durability seems to have been based partly on analogy with smallpox and yellow fever vaccines, but let me remind you that the immunity following smallpox is variable, and that the faith which the United States has in the durability of the immunity to this live vaccine is shown by the fact that I had to be revaccinated before I could come to this meeting. As you know, where there is high risk of exposure most people would recommend revaccination every year or two. The more durable immunity to yellow fever vaccine is probably related to the viremia which is associated with this vaccine. Viremia may be undesirable in an attenuated poliovirus vaccine, but may be a pre-requisite of long lasting immunity. If others find, as we did, that unfortunately the durability of antibody after feeding virus is of the same order as that following two shots of formalinized vaccines, they should not invoke a new and unproven theory of immunity to explain away the poor antigenicity of the attenuated virus vaccines. It would be wiser to rely on the results of field trials.

Methods of Evaluation

We now come to one of the most difficult problems of all, which is to devise trials that will give conclusive evidence that attenuated viruses are safe and effective. I hope that it will be clearly indicated to us how such trials can be done. This is far more important and much more difficult than measuring minor differences in *neurotropism*. Let us look at some of the ways in which trials might be conducted.

First, by orthodox controlled trials, in which one or more virus types are fed during a non-epidemic period. Because of the spread of virus from vaccinees to controls I can think of no method by which we could arrive at a precise estimate of safety and effectiveness.

To overcome the drawbacks to the orthodox controlled type of trial, attenuated viruses might be fed to the whole of one community or to one section of a city, and its subsequent experience compared with that of a distinct unprotected population. Such comparisons are unreliable. Let me give you an example of the difficulty involved in geographical comparison from Belfast. In 1957 we might have attempted to immunize the population on one side of the river and leave the other side as the control. As it happened, despite socio-economic and other similarities of the two groups, a large number of cases occurred on the east side of the river and very few on the west. If we had fed the west side early in the year we would have concluded that the vaccine was highly effective, but we might also have been open to the criticism that excreted virus had spread from the vaccinated side to the unvaccinated side and had caused an epidemic.

A third method would be to vaccinate a community and observe the paralytic attack rates over a number of years. I would be happier to see this type of trial used in veterinary medicine rather than in humans. The overall assessment of effectiveness could take some years and the assessment of safety might never be precise. Some of you may feel that if, over a period of years, the paralytic attack rate in a country was reduced by 80 or 90% it would be highly satisfactory. While this is true, if we ignore the detailed evaluation of safety we may continue to use what are really sub-optimal vaccines in the belief that they are the safest and most effective that can be developed.

Finally we must consider what we can discover by feeding virus during an epidemic in an at-



FIG. 1. Reported cases of poliomyelitis in N. Ireland from Jan.–Mid June 1957.

degree of safety by a conventional trial We have, however, also to consider the safety to the community and to territories adjacent to those where extensive programs of vaccination have been initiated Indeed I wonder if we have not reached the stage where WHO should set up a surveillance unit to watch the effect, which may of course be beneficial, on countries adjacent to those where mass feeding experiments are being done. The intentional dissemination of contagious agents on a wide scale may provide us with certain basic information which I sincerely hope will never be used except for the prevention of disease

With our present knowledge in that we do not yet know whether excreted virus = stable avirulent virus, or excreted virus = relatively stable avirulent virus, we must realize that once we have fed a population group with attenuated virus vaccines we have opened a Pandora's box You may have stopped ten cases of paralytic polio, but you may be responsible later on for paralyzing 100 children I do not think this is likely, but it is our duty to consider the worst theoretical possibilities as well as the best If poliomyelitis occurs in the contacts of non-vaccinated individuals a year after the feeding of one group of individuals can we say that they were infected with wild virus? Can we be sure that the relatively avirulent excreted virus may not have been sitting around, waiting for an opportunity to "get its teeth"? As far as possible trials must be planned in such a way that they will answer this important problem of the stability of vaccine viruses released in a community

I realize that some contribution to our knowledge of the stability of excreted vaccine viruses has been made by the human-passage studies of Smorodintsev *et al.*[18] Despite the somewhat artificial conditions which were necessary in these experiments, their results suggest that the excreted vaccine viruses are at least relatively stable. I do not think we can say more Let me draw your attention to a relevant, but rather upside-down analogy with myxomatosis of rabbits Before the first devastating epizootic of myxomatosis swept Australia, laboratory tests for virulence, including multiple passages, would have indicated that the virus was stable and that it remained uniformly lethal However, once myxomatosis was outside human control less virulent strains of virus appeared and tended to replace the more virulent original forms. Am I wrong in thinking, in the case of live poliovirus vaccines, that laboratory studies may give us the hope of stability, but that the proof must come from the field? It is the integration of all ecological features which will determine what will survive

EFFECTIVENESS

We shall doubtless be hearing about the effectiveness of oral vaccines in the field during the course of this meeting It is reasonable to judge their likely effectiveness by the antibody they induce In the laboratory-controlled studies of Verlinde *et al.*[13] using Sabin's strains, and in the Minnesota trial of Cox's viruses[1] the effectiveness was about 85 per cent as measured by antibody This is less than was obtained with three of the batches of the British formalinized poliovirus vaccine evaluated by the Medical Research Council which showed a 99 per cent effectiveness (all except two of 199 children responding with antibody to all three types).[20] The failure of attenuated vaccines to stimulate antibody in some individuals *may* of course be quite unimportant, and those who fail to respond may be the least susceptible to any poliovirus and therefore the least in need of vaccination.

Sabin has stressed the importance of the alimentary resistance to reinfection which occurs in some individuals who have been fed with attenuated strains but not in people immunized with Salk vaccine Before placing too much weight on this alimentary resistance I would like to know more about its duration and what happens when the challenge is with virulent virus and not with vaccine virus. I would like to know more about the effect of interference by other enteric viruses on the establishment of the vaccine viruses in the gut. Failure to develop antibody after feeding with attenuated strains must not be dismissed on the assumption that all those without antibody will be protected by a resistant alimentary tract

One outstanding advantage claimed for avirulent poliovirus vaccines is that a more durable immunity will be provided than with inactivated vaccines On what evidence is this based? There is evidence from Paul's studies on Eskimos[9] that a durable immunity follows infection with invasive paralytic strains of poliomyelitis, but there is no evidence that the less virulent

naturally occurring strains produce a long lasting immunity The hope of durability seems to have been based partly on analogy with smallpox and yellow fever vaccines, but let me remind you that the immunity following smallpox is variable, and that the faith which the United States has in the durability of the immunity to this live vaccine is shown by the fact that I had to be revaccinated before I could come to this meeting As you know, where there is high risk of exposure most people would recommend revaccination every year or two. The more durable immunity to yellow fever vaccine is probably related to the viremia which is associated with this vaccine Viremia may be undesirable in an attenuated poliovirus vaccine, but may be a pre requisite of long lasting immunity. If others find, as we did, that unfortunately the durability of antibody after feeding virus is of the same order as that following two shots of formalinized vaccines, they should not invoke a new and unproven theory of immunity to explain away the poor antigenicity of the attenuated virus vaccines It would be wiser to rely on the results of field trials

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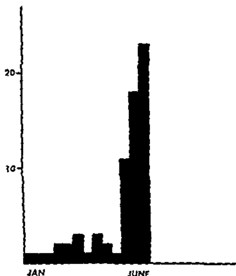


Fig 1 Reported cases of poliomyelitis in N Ireland from Jan –Mid June 1957

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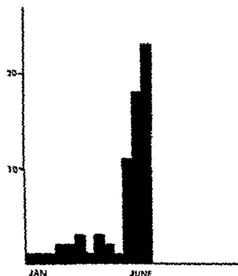


Fig 1 Reported cases of poliomyelitis in N Ireland from Jan-Mid June 1957

tempt to control it. If we feed all the susceptible members of the population with the vaccine virus we shall have to depend on alterations we produce on the epidemic curve as a measure of effectiveness.

Let me illustrate the difficulties of doing this In 1957 a Type 1 epidemic in Northern Ireland started as shown in Fig. 1.

Based on previous experience in Great Britain we predicted that the epidemic would follow the course shown in Fig. 2



FIG 2 Predicted epidemic curve with peak in August

If we had fed virus early in the epidemic we concluded that with reasonable cooperation of volunteers we might produce an epidemic curve as shown by the shaded area in Fig. 3

Indeed we did nothing, and the shaded area in Fig 3 represents exactly what did occur.

I can think of two other methods of assessing live poliovaccines during an epidemic Half the population can be fed with the virus type which is causing the epidemic or with a heterologous type. If the same virus type is fed we have no means of testing the safety of the vaccine under epidemic conditions and no way of sorting out the cases due to the wild virus and those due to the vaccine unless the excreted vaccine virus

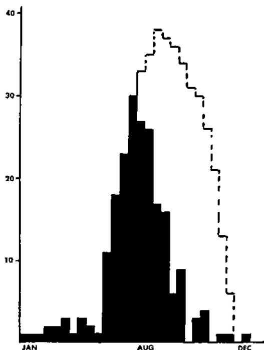


FIG 3 Reported cases of poliomyelitis 1957 N Ireland compared with predicted epidemic

has some marker If a heterologous virus type is fed, as was recently done by Dr Hale in Singapore[5] when he fed Sabin's Type 2 virus during a Type 1 epidemic, the chances of sorting things out seem very much better A complete and critical laboratory surveillance of cases following such a vaccination program is an essential part of the study It may be possible in this way to estimate the safety of a Type 2 vaccine to a community, but it must not be assumed that Type 1 vaccines will be of equal safety in similar circumstances.

One of the most important things which this Conference can do will be to define the methods whereby field trials can provide useful information. The experts on field trials with whom I have discussed the problem of assessing contagious vaccines have all considered this a most difficult problem. I cannot help feeling that when the WHO Expert Committee recommended field trials they might have given some guidance as to how they thought the trials should be conducted Perhaps we can make up for this now

Because I have attempted a critical analysis of some aspects of the live virus vaccines avail-

able at present, I would not like you to think that I have not a great admiration for the valuable work which has been done in this field I have always thought that inactivated vaccines were nearly killed by uncontrolled enthusiasm and lack of criticism. None of us want to see this happen with live virus vaccines

Let us remember in all our discussions that the development of attenuated poliovirus vaccines was originally stimulated by the need to prevent paralytic poliomyelitis in North America In many parts of the world poliomyelitis is both relatively and absolutely less important It would *be a pity, in our enthusiasm, to rid the world of* poliomyelitis if in some countries we encourage a demand for imperfect poliovirus vaccines in preference to measures for preventing other diseases of greater economic and human importance We have to face realities not all countries can afford to do all they would like in the prevention of disease

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TOPIC II. CRITERIA OF ATTENUATION, DEVELOPMENT, SELECTION, AND TESTING OF POLIOVIRUS STRAINS FOR USE IN FIELD TRIALS

1. RECENT STUDIES AND FIELD TESTS WITH A LIVE ATTENUATED POLIOVIRUS VACCINE *

DR. ALBERT B. SABIN

The Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio

DR SABIN: The past year may truly be referred to as the international live poliovirus vaccine year, since never before have investigators from so many different parts of the world combined their efforts and facilities to obtain an answer to a question of international public health importance The basic question of safety of such a vaccine for those who receive it by direct feeding as well as for those who may be immunized by contact infection could be answered only by field trials on a very large scale as was recommended in 1957 by the Expert Committee on Poliomyelitis of the World Health Organization This Committee also recommended that such large-scale field trials be carried out in countries with sufficiently large nonimmune populations where the Salk vaccine was not being used on a large enough scale to prevent a proper evaluation of the safety of the live vaccine It was because of this recommendation and also because one did not want to interfere with the maximal utilization of the already established Salk vaccine, that no attempt was made to carry out large field trials in the U.S.A

Our colleagues elsewhere in the world often in the face of great opposition, have made it possible to obtain the answer, which we in the U.S.A who developed and supplied the vaccines for international study, were unable to obtain ourselves We are meeting here now to evaluate the results of the trials that have already been completed on millions of children, as well as to consider certain basic questions about the nature and behavior of the attenuated polioviruses that have been used on such a mass scale

The status of the work up to January 1959 with the attenuated poliovirus strains that I developed was reviewed in the 14 March 1959 issue of the *British Medical Journal* In the present communication I shall present data accumulated since then on criteria of attenuation, on the role of temperature of cultivation on the selection of poliovirus particles with different degrees of neurovirulence, on monkey tests for neurovirulence of attenuated strains after prolonged propagation in the intestinal tract of vaccinees and contacts, on the persistence of immunity and resistance of the intestinal tract 2 years after feeding all 3 currently used strains to triple negative children, on interference among the 3 types of poliovirus, and on the influence of pre-existing natural infections with various enteric viruses on the multiplication of the vaccine strains in the intestinal tract Finally I shall indicate the extent to which the 3 vaccine strains that I developed have been used by various groups here present in large field trials.

Although other properties such as absent or limited capacity for multiplication in extra alimentary tract, extraneural tissues contribute

* Personal studies reported in this communication were aided by grants from The National Foundation for Infantile Paralysis, currently called The National Foundation

to the safety of an attenuated poliovirus for human beings, marked reduction in primate neurovirulence has been the most important criterion in the selection of suitable strains for human use. Absence of paralytogenic activity in monkeys inoculated intracerebrally with millions of tissue culture infective doses has been a primary requirement, and much of my work during the years of 1954 to 1956 has been concerned with the selection of strains that had the least residual paralytogenic activity on direct inoculation into the gray matter of the lumbar enlargement of the spinal cord of monkeys, because their neurons were found to be much more susceptible than those of the more highly developed chimpanzees and presumably also those of man. During the past 8 months it has become evident that the precise placement of the inoculum either in the thalamic region after intracerebral inoculation or into the gray matter of the lumbar enlargement after spinal inoculation is of the utmost importance in obtaining not only reproducible results but also valid data for establishing criteria for biologic control of vaccines for human use.

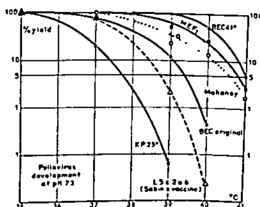
Although at the request of Doctors Cox and Koprowski I have compared by the methods that I have been using the extent of attenuation of the strains they had employed on a large scale in human beings, I will mention at this time only the results obtained with my own strains by various methods of testing. The Type 1, Type 2, and Type 3 strains have in repeated tests in my own and other laboratories produced neither paralysis nor specific lesions after intracerebral inoculation in the thalamic region, regardless of the dose inoculated. The results of spinal inoculation into the gray matter of the lumbar enlargement have varied from no paralysis, to weakness or partial paralysis and only occasionally complete paralysis of one extremity, depending on the dose and on whether the inoculum was placed largely in the posterior horn only adjacent to the anterior horn, which occurs when the needle is withdrawn too much after hitting the ventral bony surface of the spinal canal, or when it is placed too close to the mid line or too far laterally or whether it is placed largely in the ventral portion of the anterior horn, which happens when the needle is properly placed and left close to the ventral bony surface or whether it is widely distributed over many levels of the cord with associated traumatic destruction of a considerable portion of an anterior horn which is most readily accomplished by delivering a jet stream with a fine gauge needle which is moved about during the period of inoculation. I think that only those who have tried to visualize behind the skin and the bone of the small spinal canal exactly where the midline is, to estimate where the anterior horn is to place the needle in just the right place, and then examined innumerable sections to check on what was done, will have sympathy with my remarks and with the difficulties I have just mentioned. In my travels through the U.S.A. and elsewhere in the world, I found that every laboratory that I have visited has used a different method of spinal inoculation. Actually, unless people examine histologically many levels of the lumbar cord, they have no way of really checking on where they actually put the inoculum. But it should not be forgotten that it is, in effect, one of the characteristics of attenuated polioviruses that they behave in this peculiar manner, that they have to be placed in this precise position in the midst of the most susceptible neurons in the anterior horn cells to bring out their potential behavior in the lower motor neurons. But it should not be imagined that, because of the technical difficulties mentioned that the *spinal route of inoculation does not* provide further important information than that which can be obtained by the intracerebral method of testing. There is still quite a difference between a result that varies from no paralysis to weakness which sometimes is transitory, or partial paralysis only on one side, and a result in which the paralysis is extensive or complete on one side, with spread to the other side, and occasionally also affecting the back and upper extremities. The strains of all 3 types produce limited neuronal lesions in the spinal cord and their failure to multiply sufficiently to cause progressive paralysis beyond the site of inoculation remains an important criterion of attenuation. Now that the significance of different procedures of spinal inoculation is generally recognized (I hope, that like some of the things that Dr. Dick put on lantern slides, this is one of the things we can agree on), I believe that a standard method of inoculation, checked by appropriate histologic examination, should be used to provide a measure

of attenuation beyond that indicated by the intracerebral method of testing

Only during the past 6 months have we learned something of the underlying mechanism by which attenuation of the currently-used vaccine strains has been achieved Previous studies on the *in vitro* marker which has been called the *d* character (for the sake of those who may not know what the *d* character is, let me say that it has reference to the ability of the virus to form plaques under an agar overlay of reduced bicarbonate concentration), led many to believe that there was a high degree of correlation between neurovirulence for monkeys and this character However, studies that were recently completed by Dr Dulbecco and myself, show that the *d* character bears no direct relationship to neurovirulence for monkeys We started out with a triply purified plaque derivative of an attenuated Type 1 and Type 3 poliovirus, and by stepwise selective propagation under acid overlay, finally succeeded in obtaining a virus which had the same capacity to form plaques under reduced bicarbonate concentration as the most virulent poliovirus. And when the Type 1 "back mutant" was tested both intracerebrally and intraspinally in monkeys, no change in neurotropic activity was detected in the virus that had undergone a complete change in its *d* character For Type 3 on intracerebral inoculation there was absolutely no change, while on spinal inoculation the change was minimal, and I cannot be certain of its significance So it is clear that the *d* character is not directly related to neurovirulence, and the only way we could find it out was by studying "back-mutants" from triply purified plaque progeny

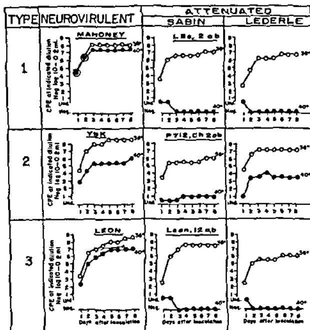
The remarkable studies of André and Marguerite Lwoff with the attenuated polioviruses that I had selected, established the fact that the genetic constitution of polioviruses determines the extent to which they may reproduce themselves in tissue culture at temperatures above and below 37°C. Studies by Lwoff and myself on mutants selected by stepwise cultivation at temperatures up to 41°C and down to 25°C established the fact that the genetic determinants for neurovirulence of Type 1 poliovirus are either identical or very closely linked with those that determine reproduction of the viruses at higher and lower temperatures. The higher the temperature at which a poliovirus can reproduce itself at a high level, the more neurovirulent it is for the monkey. Thus far, the one exception to this rule was a derivative of the Type 2, MEF_1 strain which lost much of its neurovirulence for monkeys in the process of selection for maximum neurovirulence in mice. This strain can multiply extensively in tissue culture at 39° to 40°C and its reduced neurovirulence in monkeys is apparently based on a limited affinity for the receptors of primate neurons. A mutant of Type 1 virus selected for its capacity to reproduce more extensively at 25°C than at 36°C exhibited the most limited capacity of any strain thus far tested, to produce neuronal lesions upon direct inoculation of as much as a million tissue culture infective doses into the anterior horns of the lumbar enlargement of monkeys. This 25°C mutant was not, however, completely devoid of the capacity to produce lesions at the site of inoculation of a million tissue culture infective doses A very significant observation that was made during the course of our studies on a 41°C "back-mutant" of the highly attenuated Type 1 LSc, 2ab strain (to which Dr Dick has already referred) is that while it regained full neurovirulence, it still possessed only a very limited capacity for the production of viremia and paralysis in intravenously inoculated young cynomolgus monkeys The latter is a very important character because a poliovirus may have high neurovirulence after it is put into the nervous system by the needle, but if it lacks the capacity for getting into the nervous system under natural conditions of spread, it still has not reverted to a state of high virulence

Slide 1



SLIDE 2

CYTOPATHOGENIC EFFECT IN MONKEY KIDNEY TISSUE CULTURE AT 36° AND 40°C OF NEUROVIRULENT POLIOVIRUSES AND *ATTENUATED VACCINE STRAINS*



The first slide presents data obtained by Dr Lwoff The data are based on the yield of a single cycle of multiplication of the different viruses in KB cells in suspension maintained in water bath with very carefully controlled temperatures What is shown here is that the yield of intracellular and extracellular virus—100 per cent *at 35 degrees centigrade, and the cor* responding yield of different strains at the various higher temperatures

I should like to point out that a strain of virus (KP25°) which shows a 60 per cent re*duction in yield at 37 degrees in* this type of test still gives some cytopathogenic effect (CPE) in a monolayer sheet, but the CPE is nonprogressive Even this much reduction in yield affects the release of virus and spread from cell to cell

You will notice here the different capacities or extent of reproduction at different temperatures of the different strains of known neurovirulence that were tested

Now the one exception to the rule that high reproductive capacity at 40° to 41°C is associated with high neurovirulence is shown here for the Type 2 MEF_1 *strain I obtained this strain* from Casals and "purified" it by selecting the terminal dilution progeny in newborn mice This proved to be of such reduced neurovirulence in monkeys that after intracerebral inoculation it *did not produce paralysis but it* did produce lesions. Yet this virus exhibits an even higher capacity for reproduction at 40° to 41°C than the fully virulent, Type 1 Mahoney virus

One simple test that I utilized to test the characteristics of some viruses was merely to inoculate monkey kidney tubes with different dilutions of virus from undiluted to 10-9, and then record the tubes which showed CPE These tubes were kept in an incubator with a fan because unless you have good temperature, control studies of this nature are very unreliable

The data are self-evident Although distinct differences were found between the Type 1 and Type 3 Lederle strains and my own strains as

regards neurovirulence, no difference in this test for CPE at 40°C was found. The Type 2 Lederle virus which is also the most neurotropic showed the greatest CPE at 40°C.

There is now good reason for believing that the accidental use of incubators set at 35°-36°C for the propagation of the polioviruses in tissue culture led probably to the gradual increase in particles with reduced neurovirulence for monkeys, from which the most attenuated strains were then picked by the laborious testing of the progeny of individual virus particles Low temperature mutants (25° and 30°C) of all 3 types of poliovirus are now being prepared in my laboratory for tests in human beings to determine the extent to which they may still be able to multiply in the intestinal tract The results already available indicate that no significant decrease in residual neurovirulence of the Type 1 strain occurred after a prolonged period of adaptation to satisfactory multiplication at 30°C Whether or not 25° mutants will be able to multiply satisfactorily in the human intestinal tract remains to be determined The best 25°C mutant studied thus far does not even multiply well at 36° in tissue culture

The earliest tests with attenuated poliovirus strains in human beings had shown that after many cycles of reproduction in the intestinal tract, it was possible to recover virus of somewhat greater neurovirulence upon inoculation in monkeys—much less with Types 1 and 2 than with Type 3 My own tests, as well as those of other investigators, have shown that when such virus appeared in the stools it eventually disappeared as further multiplication occurred in the same person, and no progressive increase in neurovirulence was observed either during the course of natural contact infections in families or nurs-

SLIDE 3

Cerebral Neurovirulence for Monkeys of Type 1 Polioviruses Excreted in Stools by Children, Previously Inoculated with 4 Doses of Salk Vaccine and Subsequently Fed Type 1 (LSc 2ab) Live Poliovirus Vaccine

Name	Peak titer (log_{10} TCD_{50}/gm) and days on which found	Duration of excretion days	Days after ingestion of vaccine stools tested	Paralytic effect in monkeys of log_{10} TCD_{50} in indicated range	
				8 4 - 7 1	6 4 - 5 1
O'Hara	5 2 (6 9)	27	6 13 27	0 0 0 0 0 , 0	0 0 0 , 0 0 , 0
Sehr	5 2 (6 9)	27	6 13 27	0 0 0 0 0 , 0	0 , 0 0 0 0 , 0
Rosario	5 2 (6)	27	6 14 27	0 0 0 0 0 , 0	0 , 0 0 0 0 , 0
Harris	5 2 (10)	14	7 14	0 0 0 0	0 0 0 , 0
Krugman, C	5 2 (5, 10)	13	5 13	0 0 0 , 0	0 0 0 , 0
Ortis (interfering virus first 9 days)	6 2 (20)	27	13 27	0 , 0 0 0	0 0 0 , 0
Rabinowitz	4 2 (6, 9)	9 (again at 42)	9	0 , 0	0 , 0
Keenan	3 7 (2, 9)	11	6 13	0 0 0 , 0	0 , 0 0 , 0
Troise (ECHO 7 in "pre" and 2-day stools)	3 2 (9, 20)	20	9 20	0 , 0 0 0	0 , 0 0 , 0
Bach Coxsackie A 11 in 4 to 20 day stools	2 2 (13)	13	13	0 0	0 0

eries, or during the course of 10 consecutive experimental passages of all 3 types of virus carried out in children in Leningrad by Prof. Smorodintsev. Additional studies on vaccinated children and contacts that I carried out during the past 6 months have provided further proof that a progressive increase in neurovirulence does not occur on continued propagation of my vaccine strains in the human intestinal tract. I want to emphasize the word "progressive" because it is an indication to the lack of selection of more neurotropic virus in the human intestinal tract. No living thing in nature is genetically stable except in relation to the selective media which determine favorable selection of variants that arise by mutation. Therefore, the most important question here is whether or not, the extent to which the intestinal tract actually selects variants of the ingested virus, or whether the main, progressive selection occurs in the monkeys that we inoculate. Slide 3 shows previously unpublished data on ten children who received the Type 1 vaccine. These are children who initially were not immune to Type 1 poliovirus, then had received four doses of Salk vaccine over a period of eighteen months and finally were fed the Type 1 vaccine. I tested the cultures of stools obtained during the early part, six and thirteen days, because that is the time of peak concentration when it is most likely to be transmitted, and also the last positive stool. In this particular series the results of intracerebral inoculation with both the undiluted culture fluid and the 10-2 dilution were completely negative. The next series of slides (4, 5, 6, and 7) show some of the results of studies carried out in association with Drs. Gelfand and Fox. In Louisiana, where considerable spontaneous spread of polioviruses occurs, although not as extensively as in Mexico and in some other places, they had fed these vaccine strains to one member of a family and then collected the stools from the index child and also from the susceptible contacts, and I studied the neurovirulence of the excreted viruses in monkeys. Slide 4 shows

SLIDE 4

Cerebral Neurovirulence of Type 1 Viruses Excreted by Index Child Who was Fed Type 1 Vaccine (LSc 2ab) and by Contact Children in Family 125

Person tested	Days after ingestion of vaccine by index child stool tested	Log_{10} TCD_{50} of virus inoculated in monkeys and result
Index child	7	7 9 / 0 0 5 9 / 0,0
	13	7 9 / 0,0 5 9 / 0,0
Contact - Age 1	12	8 5 / 0,0 6 5 / 0,14 (part)
First positive stool 10 days	28	7 4 / 0 0 5 7 / 0 0
Contact - Age 7	6	8 2 / 0 0 6 2 / 0 0
First positive stool 6 days	12	7 6 / 7 7S 5 9 / 7S, 8x 5 0 / 0,0 4 0 / 0 0 3 0 / 0 0 2 0 / 0,0
	28	8 2 / 0,0 6 2 / 0 11 (part)

0 = no paralysis. 14 part = partial paralysis 14 days after inoculation, monkey observed for 28 days. 7S = initial paralysis 7 days after inoculation, monkey sacrificed for quantitative test for virus in spinal cord and zone phenomenon found.

regards neurovirulence, no difference in this test for CPE at 40°C was found. The Type 2 Lederle virus which is also the most neurotropic showed the greatest CPE at 40°C

There is now good reason for believing that the accidental use of incubators set at 35°-36°C for the propagation of the polioviruses in tissue culture led probably to the gradual increase in particles with reduced neurovirulence for monkeys, from which the most attenuated strains were then picked by the laborious testing of the progeny of individual virus particles Low temperature mutants (25° and 30°C) of all 3 types of poliovirus are now being prepared in my laboratory for tests in human beings to determine the extent to which they may still be able to multiply in the intestinal tract The results already available indicate that no significant decrease in residual neurovirulence of the Type 1 strain occurred after a prolonged period of adaptation to satisfactory multiplication at 30°C. Whether or not 25° mutants will be able to multiply satisfactorily in the human intestinal tract remains to be determined. The best 25°C mutant studied thus far does not even multiply well at 36° in tissue culture

The earliest tests with attenuated poliovirus strains in human beings had shown that after many cycles of reproduction in the intestinal tract, it was possible to recover virus of somewhat greater neurovirulence upon inoculation in monkeys—much less with Types 1 and 2 than with Type 3. My own tests, as well as those of other investigators, have shown that when such virus appeared in the stools it eventually disappeared as further multiplication occurred in the same person, and no progressive increase in neurovirulence was observed either during the course of natural contact infections in families or nurs-

SLIDE 3

Cerebral Neurovirulence for Monkeys of Type 1 Polioviruses Excreted in Stools by Children Previously Inoculated with 4 Doses of Salk Vaccine and Subsequently Fed Type 1 (LSc 2ab) Live Poliovirus Vaccine

Name	Peak titer ($\log_{10}$ TCD_{50}/gm) and days on which found	Duration of excretion days	Days after ingestion of vaccine stools tested	Paralytic effect in monkeys of $\log_{10}$ TCD_{50} in indicated range	
				8 4 - 7 1	6 4 - 5 1
O'Hara	5 2 (6, 9)	27	6 13 27	0, 0 0, 0 0, 0	0, 0 0, 0 0, 0
Sehr	5 2 (6 9)	27	6 13 27	0 0 0 0 0, 0	0, 0 0 0 0, 0
Rosario	5 2 (6)	27	6 14 27	0 0 0 0 0, 0	0 0 0 0 0, 0
Harris	5 2 (10)	14	7 14	0 0 0 0	0 0 0 0
Krugman C	5 2 (5, 10)	13	5 13	0 0 0, 0	0 0 0, 0
Ortis (interfering virus first 9 days)	6 2 (20)	27	13 27	0 0 0, 0	0 0 0, 0
Rabinowitz	4 2 (6, 9)	9 (again at 42)	9	0 0	0 0
Keenan	3 7 (2, 9)	13	6 13	0 0 0 0	0, 0 0, 0
Troise (ECHO 7 in "pre" and 2-day stools)	3 2 (9, 20)	20	9 20	0, 0 0 0	0 0 0 0
Bach Coxsackie A 11 in 4 to 20 day stools	2 2 (13)	13	13	0, 0	0 0

families in which the Type *2* vaccine was fed The pattern is rather similar to that reported for children who were fed the Type 1 vaccine before. An occasional monkey, frequently one receiving the lower dose, indicative of zoning, develops paralytic manifestations after inoculation. The asterisk has reference to the fact that no cytopathogenic virus was recovered from the spinal cord of this paralyzed monkey Family 103 is of particular interest because it shows what may happen under field conditions The index *child in this family yielded no stools with* intracerebral activity in monkeys, but one of the contact children at two days after the index child was fed already was excreting a thousand times more virus per gram of stool than the index child We are very suspicious that this child might have picked up the virus from a naturally infected person and not from the index child.

Fortunately, among the Type 2 viruses there are differences in the antigenic constitution For example, the antiserum that we have against the vaccine strain readily neutralized 10 million infective doses of the vaccine strain, but the strain recovered from this contact was not as readily neutralized by this serum as the strain or the virus excreted by the index child Nevertheless, the virus excreted at two days by this contact was one of *those viruses of very low neurovirulence* encountered in nature In nature there are "good" viruses, "intermediate" viruses, "slightly bad" viruses, 'bad" virsuses, and "very bad" viruses. It is a continuous spectrum.

Upon further multiplication of this "wild" Type 2 virus in the intestinal tract a pattern

Slide 6

Cerebral Neurovirulence of Type 3 Polioviruses Excreted by Familial Contacts of Children Who Were Fed Type 3 Vaccine (Leon $12a_1b$)

Family	Person tested	Days after ingestion of vaccine by index child stool tested	Log_{10} TCD_{50} of virus inoculated in monkeys and result
110	Contact - Age 5 First posit stool 2 days	12	7 9 / 0,0 ; 6 2 / 0 22 P
	Contact - Age 2 First posit stool 2 days	13	7 1 / 0 9 ; 5 4 / 0 11 part ; 4 0 / 0 0
	Contact - Age 6 First posit stool 6 days	13	7 9 / 0,0 ; 6 2 / 0 0
	Contact - Age 9 First posit stool 7 days	15	8 2 / 0,0 ; 6 5 / 18 B, 19 P al ; 5 0 / 0,0 ; 4 0 / 0 0
115	Contact - Age 1 First posit stool 5 days	23	7 1 / 0m, 0m 0m, 0m ; 5 4 / 0 24 ; 5 0 / 0 0 ; 4 0 / 0 0
116	Contact - Age 2 First posit stool 6 days	16	7 1 / 8 10 ; 5 4 / 15 0 ; 5 0 / 0 13 transit ; 4 0 / 0,0
	Contact - Age 4 5 First posit stool 11 days	17	7 7 / 0,0 ; 6 0 / 0 0
116 A	Contact - Age 6 First posit stool 7 days or earlier	21	7 9 / 0 0 ; 6 2 / 0,12 ; 5 0 / 0 0

a study on a Type-1 family In one of the contacts (age 7), the greatest change that I have hitherto encountered with Type 1 virus in excreted stool has taken place But let's analyze what happened in this family. The stool excreted by the index child 7 and 13 days after feeding yielded cultures which were not paralytogenic for intracerebrally inoculated monkeys even with doses of 80 million TCD_{50}. Contact, age one, who first became positive at ten days after feeding of the index child two days later yielded a stool culture which in a dose of 300 million TCD_{50} was not paralytogenic in 2 monkeys, but one of the two monkeys inoculated with the three million dose had a partial paralysis on the fourteenth day But on continued multiplication of the virus in this child, in the culture of the stool obtained at 28 days no paralytogenic effect was observed While the number of monkeys used is small it is nevertheless clear that no progressive increase in neurovirulence of the virus occurred with prolonged propagation in the intestinal tract. The other contact (age 7) started out at six days with a pattern similar to that of the index child. About six days later after further multiplication in its intestinal tract, the monkeys inoculated with $10^{7.6}$ TCD_{50}, and $10^{7.9}$ TCD_{50} exhibited paralytic manifestations. A zone phenomenon was found in testing for virus in the spinal cord, which means that the original ten per cent extract produced no CPE while higher dilutions did With smaller concentrations of virus no paralytogenic effect was produced, and the test on the 28-day stool shows not only that there was no progressive increase in neurovirulent virus on further multiplication in this child, but rather the reverse—doses of about 100 million TCD_{50} were not paralytogenic

Slide 5 shows a series of tests on contacts of

SLIDE 5

Cerebral Neurovirulence of Type 2 Polioviruses Excreted by Familial Contacts of Children Who Were Fed Type 2 Vaccine (P712,Ch,2ab)

Family	Person tested	Days after ingestion of vaccine by index child stool tested	Log_{10} TCD_{50} of virus inoculated in monkeys and result					
131 A	Contact - Age 7 First posit stool 14 days	21	7 4 / 0,0	6 2 / 0,0				
134	Contact - Age <1 First posit stool 5 days	15	7 7 / 0,0	6 0 / 0 11*				
	Contact - Age 7 First posit stool 11 days	25	7 1 / 0,0	5 4 / 0,10	5 0 / 0,0	4 0 / 0,0		
	Contact - Age 6 First posit stool 14 days	25	7 1 / 0,5	5 4 / 0 Facial only	5 0 / 0 0	4 0 / 0,0		
103	Index child	2	7 7 / 0,0	5 7 / 0,0				
		18	7 7 / 0,0	5 7 / 0 0				
	Contact** - Age 2 First posit stool 2 days	2	7 7 / 0,4	5 7 / 0,0				
		18	7 7 / 7 9	6 0 / 7 7	5 0 / 0,0	4 0 / 0 13 sl	3 0 / 0 13 part	2 0 / 0,0
		41	6 9 / 0,10 slight transit	4 9 / 0,0				

SLIDE 8

TYPE 1 POLIOVIRUS

Comparative Cytopathogenic Activity in Monkey Kidney Tissue Cultures at 36° and 40°C of Fully Neurovirulent Virus of the Highly Attenuated Derivative in Oral Vaccine, and of Virus in Stools Excreted by Children after Ingestion of Vaccine

VIRUS TESTED	LOG_{10} TCD_{50}/ml or gm.		PARALYTIC ACTIVITY in MONKEYS	
	36°C	40°C	CEREBRAL	SPINAL
MAHONEY	6 7	8 2	10^0-10^1	
ORAL VACCINE LSc 2ab	7 7	Undiluted Slight	Negative at 10^{8}	Occasional at 10^7 and 10^5 Neg at 10^3
"D S"-7 Days After Vaccine Orig Stool / Stool Culture	5 2 / 7 7	<1.0 / Undiluted Slight	Negative at $10^{7.4}$	Negative at $10^{3.7}$
A S -20 Days After Vaccine Orig Stool / Stool Culture	4 7 / 7 7	<1.0 / Undiluted incomplete	1/4 at $10^{2.4}$	Positive at $10^{2.4}$

SLIDE 9

TYPE 2 POLIOVIRUS

YSK	9 2	6 7	10^0-10^1	
ORAL VACCINE P712 Ch, 2ab	7 3	1 7 partial	Negative at $10^{7.7}$	Occasional at $10^{6.7}$ Negative at $10^{3.7}$
D S.-9 Days* After Vaccine Orig Stool / Stool Culture	4 7 / 7 7	<1.0 / Undil-partial	Negative at $10^{7.7}$	Negative at $10^{5.7}$
A S -7 Days After Vaccine Orig Stool / Stool Culture	4 7 / 7 7 (8.4)	<1.0 / 2 7 partial	Negative at $10^{8.4}$	Positive at $10^{6.4}$
A S -56 Days After Vaccine Orig Stool / Stool Culture	5 2 / 7 7	<1.0 / 2 2 partial	Negative at $10^{7.2}$	Negative at $10^{6.5}$

* This child developed pneumonitis within 24 hours after the vaccine and had temperatures reaching 38°-40° Centigrade for several days

"wild" polioviruses has been so low that they had remained triple negatives up until the time they were fed the live virus vaccines at ages 5 to 11 years.

The capacity of the virus in the original stools and in the stool cultures to produce a CPE at 40°C is correlated with any changes in neurovirulence as tested in monkeys I purposely selected for each type examples of stools without any demonstrable change in neurovirulence and those which had exhibited the greatest change The most striking finding in the tests with the Types 1 and 2 strains is that the specimens

SLIDE 10

TYPE 3 POLIOVIRUS

Comparative Cytopathogenic Activity in Monkey Kidney Tissue Cultures at 36° and 40°C of Highly Virulent Virus of the Highly Attenuated Derivative in Oral Vaccine and of Virus in Stools Excreted by Children After Ingestion of Vaccine

VIRUS TESTED	$LOG_{10}TCD_{50}$/ml or gm		PARALYTIC ACTIVITY in MONKEYS	
	36°C	40°C	CEREBRAL	SPINAL
LEON	8 7	7 7	Highly neurovirulent	
ORAL VACCINE LEON 12a,b	8 2	Undiluted Slight	Negative at $10^{7.8}$	Occasional at $10^{5.5}$ Negative at $10^{3.5}$
D S -9 Days After Vaccine Orig Stool / Stool Culture	3 7 (4 7) / 6 7 (8 2)	<1 0 / 2 2 partial	1/3 at $10^{5.4}$ / Irreg at $10^{4.5}$-$10^{3.2}$	
D S - 21 Days After Vaccine Orig Stool / Stool Culture	2 7 (3.7) / 7 2	<1.0 / 2.2 partial	1/4 at $10^{8.4}$ / Irregular at $10^{5.4}$-$10^{4.4}$ / Negative at $10^{3.4}$	
S F -13 Days After Vaccine Orig Stool / Stool Culture	5 7 / 7 7	3 7 / 5 7 partial	Negative at $10^{4.8}$ / Irreg at 10^{5}-$10^{3.7}$	
S F - 20 Days After Vaccine Orig Stool / Stool Culture	6 7 / 6.2 (7.4)	3 2 / 3 2 partial	Negative at $10^{7.4}$	

which exhibited the greatest change in the monkey test did not differ from the vaccine strains in their inability to produce CPE at 40°C

In the Type 2 test, one child is of particular interest, because within 24 hours after feeding the Type 2 vaccine, the child developed a pneumonitis and had temperatures of 38°-40° for several days Unfortunately, no stools were collected during the period of the fever, and we do not know whether or not the multiplication of virus was suppressed during that period But stools collected later showed that there was no interference with virus multiplication, and that the virus which multiplied showed no difference in its failure to reproduce at 40°, and, furthermore, the tests for neurovirulence showed no change at all in this particular instance

For Type 3, the relationship between capacity for multiplication at 40°C and neurovirulence as tested in monkeys is not as cleancut as for Types 1 and 2

I will not analyze this here in detail, except to point out that a change in reproductive capacity at 40°C to the extent that there was partial cytopathogenic effect, as compared to no effect at all with the original vaccine, was not correlated with increased activity in neurovirulence tests What is of special interest, however, is that the virus in the stools of "D S," which exhibited the greatest change in the monkey tests observed by any-

similar to that I have previously reported to occur in some children fed the Type 3 vaccine was observed here, at 18 days there was definitely an increase, although partial and slight in the lower concentrations of the activity on intracerebral inoculation in monkeys, but at 41 days this also did not persist

Slides 6 and 7 show some results with Type 3 As I have indicated previously, we have had more frequent evidence of greater modification of the Type 3 virus, than of Types 1 and 2 In general the results obtained with the viruses excreted by the contacts are no different from those obtained previously with the stools of persons originally fed the Type 3 vaccine In slide 6 the designation 0ᵐ has reference to a mononuclear *meningitis in the monkey. The* responsible agent was not cytopathogenic We have not identified it, but it was not polio

Slide 7 shows an analysis of what happened in a single family The data on the index child provide an example of the appearance and disappearance of virus of increased neurotropism Similar evidence for disappearance of virus of slightly increased neurotropism can also be seen in the contacts

This phenomenon, which has been observed by Verlinde and Smorodintsev as well as myself is the basis for my statement that there is no progressive selection of virus of increased neurotropism in the human alimentary tract

Slides 8, 9, and 10 show some tests on virus that was excreted by children under home conditions in which the possibilities for exposure to

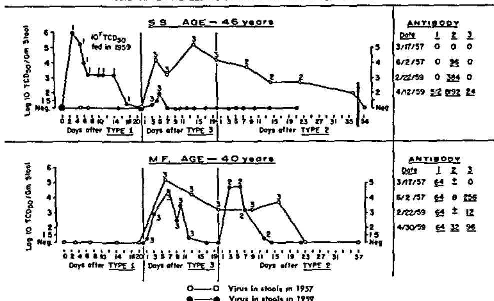
SLIDE 7

Cerebral Neurovirulence of Type 3 Polioviruses Excreted by Index Child Fed Type 3 Vaccine (Leon 12 a_1b) by its Familial Contacts and by Other Children Who Were Exposed to Familial Contacts but not to Index Child

Group	Person tested	Days after ingestion of vaccine by index child stool tested	Log_{10} TCD_{50} of virus inoculated in monkeys and result			
Family 121 A	Index child	2	7 9 / 0,0	5 9 / 0 0		
		26	7 9 / 0,10 (sl tr)	5 9 / 12 17 (sl tr)	5 0 / 0,0	4 0 / 0 0
		52	8 7 / 0 0	6 7 / 0 0		
	Contact - Age 10 First posit stool 4 days	4	8 0 / 0 10	6 0 / 0,0		
		29	7 4 / 16, 16 (sl tr, part)	5 4 / 0 0		
		54	8 1 / 0,0	6 1 / 0,0		
	Contact - Age 8 First posit stool 5 days	5	8 4 / 0,10	6 4 / 0 0		
		29	7 9 / 0 0	5 9 / 0 0		
		52	7 9 / 0	5 9 / 0,20 part ,slight		
	Contact - Age <1 First posit stool 10 days	15	8 2 / 0 10 (part)	6 2 / 0 18 (transit)		
		29	8 4 / 0,0	6 4 / 0 0		

Slide 12

MULTIPLICATION OF VIRUS IN ADULTS WITH VARIOUS PRE-EXISTING PATTERNS OF IMMUNITY AFTER INITIAL FEEDING OF 3 TYPES OF POLIOVIRUS VACCINE STRAINS AT 3-WEEK INTERVALS AND AFTER REFEEDING IN SAME MANNER 2 YEARS LATER



Slide 13

STATUS OF NEUTRALIZING ANTIBODY AND RESISTANCE OF INTESTINAL TRACT 2 YEARS AFTER FEEDING TYPE 1 POLIOVIRUS VACCINE STRAIN, LSc, 2ab

Antibody Prior to Feeding in 1957 (I II III)	Name	Age Years	Result of Feeding in 1957: Multiplication of Virus — Duration Days	Result of Feeding in 1957: Multiplication of Virus — Peak Titer TCD_{50}/Gm. Stool Log 10	Result of Feeding in 1957: Antibody pH	Antibody Prior to Refeeding in 1959	Result of Refeeding in 1959: Antibody pH	Result of Refeeding in 1959: Multiplication of Virus — Days Positive	Result of Refeeding in 1959: Multiplication of Virus — Peak Titer TCD_{50}/Gm Stool Log 10
0 0 0	1 AS	5	28	6 2	2048	4096 3072	3072	None	-
	2 DS	7	21	5 2	4096	8192 , 3072	3072	only 4th	1 3
	3 KF	15	21	5 2	2048	1024 , 384	384	None	-
	4 SF	5	21	6 2	4096	2048 , 1536	3072	only 8th-9th	4 5
	5 PF	9	21	5 2	4096	2048 , 1024	4096	4 - 13	4 2
	6 SS	46	None	-	<4	<4	512	3 - 17 Fed 10^7	6 0
0 0 +	7. HF	34	42	5 2	512	1024 , 1024	768	None	-
8/0 + + Salk Vaccine	8 JR	27	14	5 2	2048/700	1024/256	1024/1400	3 - 9	3 5
0 + +	9 HK	21	10	3 7	512	256			
	10 AM	21	14	4 3	4096	3072			

one thus far, exhibited no CPE at 40°C in the original state, and even after propagation in tissue culture differed only slightly from the original vaccine strain in its reproductive capacity at 40°C.

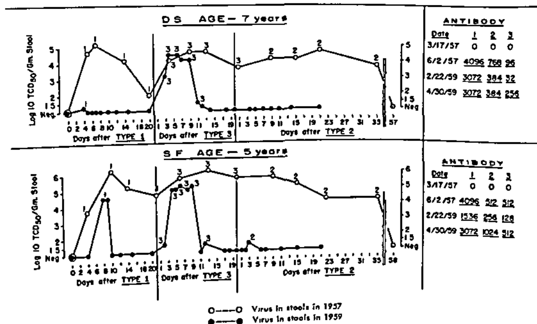
In the light of our newer knowledge about the role that higher temperatures can play in favoring the growth of polioviruses with somewhat increased capacity for multiplication in the monkey nervous system, it appears possible that during the course of multiplication in the human intestinal tract at 37° to 38°C some virus particles appear which have a greater capacity for multiplying in monkeys that have a normal temperature range of 38° to 40°C. Such an event would be conducive to further selective propagation of more neurovirulent particles in the monkey nervous system, and the end result would in no way reflect the actual characteristics of the viral population in the human intestinal tract

Since natural infections with polioviruses give rise to long-lasting immunity, and since the live poliovirus vaccine strains reproduce the intestinal phase of the natural infection, there is some basis for expecting that they may also produce long-lasting immunity I want to add here, *not necessarily*, because the extent to which it multiplies, the duration of multiplication, and many other factors may play a role.

I have previously reported what happens when small doses of virus multiply for a short period of time—the antibody often fails to persist and resistance is at best only partial. Five triple-negative children who received the 3 currently used vaccine strains at 3-week intervals early in 1957, and 6 adults who received one or more types early in 1957, were tested 2 years later and no significant change was found in their antibody titers. For the record I should like to say that the 1957 sera were stored in a deep freeze and all comparative tests were carried out simultaneously on the 1957 and 1959 sera. The 5 children and 4 of the adults were again fed about 500,000 to 1 million tissue culture infective doses of the vaccine strains, and tests for multiplication of the virus were carried out as a measure of the resistance of the intestinal tract to reinfection.

Slide 11

VIRUS MULTIPLICATION IN TRIPLE NEGATIVE CHILDREN AFTER INITIAL FEEDING OF 3 TYPES OF POLIOVIRUS VACCINE STRAINS AT 3-WEEK INTERVALS AND AFTER REFEEDING IN SAME MANNER 2 YEARS LATER



of 9, and in the ninth there was only minimal viral multiplication.

This should be kept in mind for comparison with the results that I am now to report on the children who had the three types consecutively at three-week intervals two years ago, and then were refed No virus multiplication was detected in 4 of the 7 who received the Type 1 vaccine, and the other 3 had limited multiplication. All 8 who were tested with the Type 2 vaccine exhibited complete or almost complete resistance Among the 8 persons who were fed the Type 3 vaccine, significant virus multiplication occurred for a period of about 10 days in 7, and the only person to exhibit marked resistance to reinfection was an adult who failed to develop demonstrable antibody after a 3-week period of virus multiplication in 1957 These results are comparable to those previously obtained in persons with naturally acquired immunity, in which the incidence of reinfection with the Type 2 virus was much higher than with Types 1 or 2 These studies supplied additional evidence that the resistance of the intestinal tract to reinfection bears no relationship to the level of antibody in the blood. The high incidence of resistance to reinfection with the Types 1 and 2 viruses upon the feeding of such large doses 2 years after the original oral vaccination provides some basis for the expectation that mass vaccination by the oral route may ultimately result in breaking the chain of transmission of these naturally occurring polioviruses, and thus terminate their coexistence with man since earliest evolutionary times It is well to bear in mind that in order to break the chain of transmission, you do not have to have everybody immune Even if resistance of the intestinal tract occurred only in a large proportion of individuals there is reason to look for—I don't know that it will happen—a break in the chain of transmission of the virus

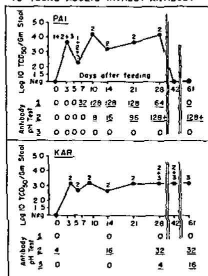
The recommended administration of the 3 types of vaccine virus separately in the order of 1, 3, and 2, at intervals of 4 weeks or longer, is based on the demonstration of frequent partial or complete interference with multiplication of one or two types when a mixture of all 3 or mixtures of any two types are fed at one time (Slides 16 to 19). I have not had an opportunity to publish these data before, or to show the basis on which I had recommended that the particular vaccine strains that I have studied had best be administered in the order that was recommended· 1, 3, and 2

I would like to say that the data that I am about to show were not the only reason for recommending that the individual types be given separately If you give only one type at a time in a field trial, you at least have two chances out of three in checking on whether a given infection with poliovirus was caused by the virus that was fed or by a naturally occurring poliovirus. If you feed all three types simultaneously, you have no opportunity for checking

Slide 16 shows a detailed analysis of virus multiplication and antibody development in a triple negative young adult who was fed a mixture of all 3 types of poliovirus Volunteer

Slide 16

EFFECT OF FEEDING MIXTURE OF TYPES 1, 2, AND 3 POLIOVIRUS VACCINE STRAINS
(10[illegible] PFU each of LSc,2ab, P712,Ch,2ab, and Leon 12a,b)
TO YOUNG ADULTS WITHOUT ANTIBODY



"PAI" excreted all 3 types for the first 6 days and after that only Type 2

Now, what happened to his antibody? Please note that he did develop Type 1 antibody in the usual time, i e in seven days, reaching a titer of 128 at 21 days—but at 61 days he no longer had demonstrable Type 1 antibody In this case a serologic test alone at 4 weeks would have indicated that there was no interference with Type 1 but both the virus studies and the serologic

Slide 14

STATUS OF NEUTRALIZING ANTIBODY AND RESISTANCE OF INTESTINAL TRACT 2 YEARS AFTER FEEDING TYPE 2 POLIOVIRUS VACCINE STRAIN, P712, Ch, 2ab

Antibody Prior to Feeding in 1957 I II III	Name	Age Years	Result of Feeding in 1957 — Multiplication of Virus: Duration Days	Result of Feeding in 1957 — Multiplication of Virus: Peak Titer TCD_{50}/Gm Stool Log 10	Result of Feeding in 1957 — Antibody pH	Antibody Prior to Refeeding in 1959	Result of Refeeding in 1959 — Antibody pH	Result of Refeeding in 1959 — Multiplication of Virus: Days Positive	Result of Refeeding in 1959 — Multiplication of Virus: Peak Titer TCD_{50}/Gm Stool Log 10
0 0 0	1 AS	5	56+	5 2	512	256 , 128	256	only 9th-11th	1 3
	2 DS	7	35	4 7	768	768 , 384	384	None	-
	3 KF	11	21	4 2	1024	768 , 384	768	None	-
	4. SF	5	35	5 7	512	512 , 256	1024	only 3rd	1 5
	5 PF	9	55+	5 7	512	384 , 256	512	None	-
	6 SS	46	36	3 7	256 , 96	384	8192*	None	-
0 0 +	7 HF	34	57+	4 2	256	192 , 96	128	None	-
	8 WP	24	21	4 7	96	96			
+ ± 0	9 MF	40	None	-	8	4 , <4	32	3 - 12	4 2

Slide 15

STATUS OF NEUTRALIZING ANTIBODY AND RESISTANCE OF INTESTINAL TRACT 2 YEARS AFTER FEEDING TYPE 3 POLIOVIRUS VACCINE STRAIN, LEON, $12a_1b$

Antibody Prior to Feeding in 1957 I. II III	Name	Age Years	Result of Feeding in 1957 — Multiplication of Virus: Duration Days	Result of Feeding in 1957 — Multiplication of Virus: Peak Titer TCD_{50}/Gm Stool Log 10	Result of Feeding in 1957 — Antibody pH	Antibody Prior to Refeeding in 1959	Result of Refeeding in 1959 — Antibody pH	Result of Refeeding in 1959 — Multiplication of Virus: Days Positive	Result of Refeeding in 1959 — Multiplication of Virus: Peak Titer TCD_{50}/Gm Stool Log 10
0 0 0	1 AS	5	14	4 7	128	256 , 96	384	2 - 10	5 0
	2. DS	7	20	4 7	96	96 32	256	3 - 12	4 5
	3 KF	11	20	5 2	384	128 64	512	3 - 12	5 0
	4 SF	5	20	4 2	512	256 128	512	2 - 12	5 2
	5 PF	9	23	5 7	96	64 32	256	7-10,<17	5 2
	6 SS	46	20	5 2	<4	<4	24	3 - 5	2 0
+ ± 0	7 MF	40	36	5 2	256	16 12	96	3 - 12	4 5
0 0 ± (12)	8 HF	34	7th-13th	Trace Together with I	16	>64 128	128	3 - 12	4 2

For those who have not read my previous report in the (14 March 1959) *British Medical Journal*, previous tests on Type 1 showed that with only one exception there was complete resistence to reinfection at about 9 to 15 months These were adult volunteers With Type 2, excepting one individual who had been fed two types of virus simultaneously previously, there was also complete resistance With Type 3, at 8 to 15 months, there was complete resistance in 8 out

SLIDE 19

EFFECT OF FEEDING MIXTURE OF TYPE 2 AND TYPE 3 POLIOVIRUS VACCINE STRAINS ($10^{5.5}$ PFU EACH OF P712, Ch, 2ab AND Leon, 12 a,b) TO YOUNG ADULTS WITHOUT ANTIBODY FOR BOTH OF THESE TYPES

Slide 19 shows data on four individuals who lacked antibody for 2 and 3. They were fed the mixture of 2 and 3 It shows the different patterns that can happen in different individuals. In "CRA" both types were excreted during the first 5 days, while Type 2, the dominant one, was still being excreted in trace amounts at 61 days. You will notice that the small amount of multiplication of Type 3 did not lead to the development of Type 3 antibody; only Type 2 antibody appeared

In "ASH", even though the Type 3 virus was found only at 3 days, Type 3 antibody did develop, but it disappeared in 61 days The Type 2 again was the dominant one.

In "HAA" and "GAR" both types multiplied together for 14 and 21 days respectively In one case, Type 2 persisted, and in the other case, Type 3 persisted for a longer period, and they developed antibodies for both

It is evident that the actual course of events can be appreciated only by careful studies for virus multiplication and antibody formation and persistence in individuals originally lacking the antibodies corresponding to the mixtures of the types of vaccine fed and living under conditions which preclude contact infections Studies involving only antibody tests on children or adults living under conditions conducive to natural transmission of infection with all 3 types, such as can obtain when a mixture of all 3 types is fed in family groups or institutions to persons of whom some are immune to only one or two types, cannot give an accurate picture of the course of events To the best of my knowledge, the available information indicates that the administration of mixtures of these vaccine strains cannot *be expected to yield the maximum immunogenic* effect, except under conditions in which ample opportunities for contact infection prevail.

During the course of a study at a school for mentally retarded children in New York in March 1958, we discovered that abnormal patterns of multiplication of the orally administered vaccine strains were invariably associated with concurrent spontaneous infections with various enteric viruses—ECHO, Coxsackie A and B, adenoviruses and a new type of enterovirus that can be detected in cultures of "human" cells but not of monkey kidney cells or in newborn mice This interference exhibited itself in several ways complete suppression of detectable multi-

test at 2 months clearly showed the limited multiplication of the Type 1 virus and the transitory antibody response The Type 2 antibody persisted Please note also that, despite the presence of apparently small amounts of Type 3 virus in the stools for 6 days, he developed no antibody for Type 3 virus.

In the second person (KAR) it was difficult to decide what his very low level of antibody Type 2 virus meant in the absence of any Type 1 and Type 3 antibody. At any rate, when all three types were fed to him, only Type 2 virus was detected in his stools for the first three weeks, Type 2 and Type 3 were present at 4 and 6 weeks, and after that only Type 3 virus. No Type 1 virus was found in his stools and he developed no Type 1 antibody The Type 2 antibody increased in titer. Only after the Type 3 virus began multiplying some Type 3 antibody appeared.

This test was carried out under conditions in which, over a period of three years, I had never observed contact infection These are quarantined young adults in a federal reformatory They had no contact with children, and during all the studies there over a period of years I have never found spontaneous poliomyelitis infection, nor have I found any spread of virus from one vaccinated person to another.

This leads me to wonder whether in this particular individual the Type 3 virus may not have actually been "latent" or multiplied only at a "subdetectable" level during the first 3 weeks.

Slide 17 shows what happened in two individuals who had only naturally acquired Type 3 antibody, but lacked 1 and 2; they were fed Type 1 and Type 2

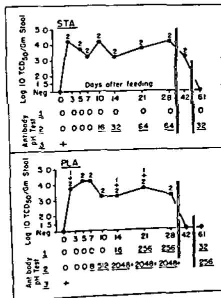
In the first individual (STA) the Type 1 virus was completely suppressed. Only Type 2 virus multiplied. He developed no Type 1 antibody, while the Type 2 antibody response was of the usual order

In the second individual (PLA) Type 1 virus was found only on the 2nd, 14th and 21st days, while Type 2 virus was recovered during the entire period of 28 days. These data again point to the dominance of this particular strain of Type 2 virus, although in this case a satisfactory antibody response developed to both types

It is in a situation such as this that I found subsequent resistance of the intestinal tract to Type 1 virus to be only partial

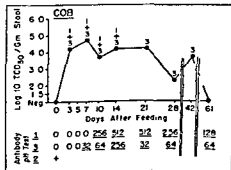
Slide 18 shows an individual who had no antibody for Types 1 and 3 and was fed a mixture of these two viruses. In his case both types multiplied for a period of fourteen days, but Type 3, the dominant one, continued to multiply by itself for at least another 4 weeks. Antibody developed to both types

Slide 17



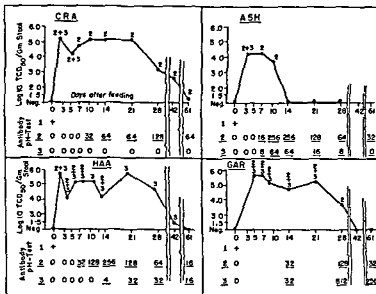
Slide 18

EFFECT OF FEEDING MIXTURE OF TYPE 1 AND TYPE 3 POLIOVIRUS VACCINE STRAINS ($10^{8.8}$ PFU each of LSc, 2ab and P712, Ch, 2ab) TO YOUNG ADULT WITHOUT ANTIBODY FOR BOTH OF THESE TYPES



SLIDE 19

EFFECT OF FEEDING MIXTURE OF TYPE 2 AND TYPE 3 POLIOVIRUS VACCINE STRAINS ($10^{5.4}$ PFU EACH OF P712, Ch, 2ab AND Leon, 12 a,b) TO YOUNG ADULTS WITHOUT ANTIBODY FOR BOTH OF THESE TYPES



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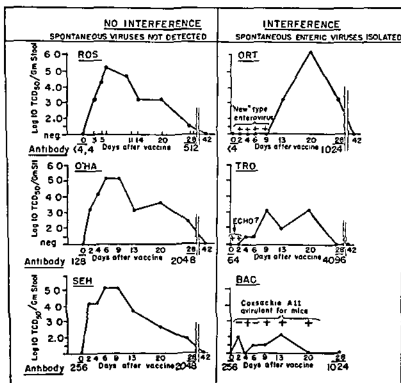
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During the course of a study at a school for mentally retarded children in New York in March 1958, we discovered that abnormal patterns of multiplication of the orally administered vaccine strains were invariably associated with concurrent spontaneous infections with various enteric viruses—ECHO, Coxsackie A and B, adenoviruses and a new type of enterovirus that can be detected in cultures of "human" cells but not of monkey kidney cells or in newborn mice This interference exhibited itself in several ways complete suppression of detectable multi-

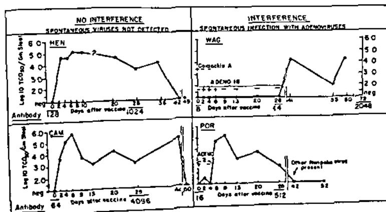
SLIDE 20

PATTERNS OF INTERFERENCE WITH MULTIPLICATION OF INGESTED TYPE 1 VACCINE POLIOVIRUS BY SPONTANEOUS INFECTIONS WITH VARIOUS ENTERIC VIRUSES IN CHILDREN WITHOUT HOMOTYPIC ANTIBODY PRIOR TO INOCULATION OF 4 DOSES OF SALK VACCINE

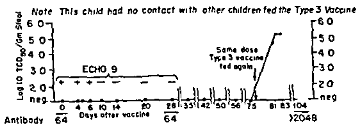


SLIDE 21

PATTERNS OF INTERFERENCE WITH MULTIPLICATION OF INGESTED TYPE 3 VACCINE POLIOVIRUS BY SPONTANEOUS INFECTIONS WITH ADENOVIRUSES IN CHILDREN WITHOUT HOMOTYPIC ANTIBODY PRIOR TO INOCULATION OF 4 DOSES OF SALK VACCINE



SLIDE 22



plication for varying periods of time followed by active multiplication of the orally administered vaccine strain or by a continued low level of multiplication, such as would not be detectable in tests on the minute amounts of stool obtainable on rectal swabs Antibody developed in all children who lived in contact with others who were fed the vaccine strains. In one child, who lived apart from the others, a spontaneous ECHO Type 9 virus infection completely interfered with the multiplication of the Type 3 poliovirus that was fed and no antibody formed The susceptibility of this child's intestinal tract to Type 3 poliovirus was proved by feeding the same dose of vaccine virus 11 weeks later, which was followed by both extensive virus multiplication and antibody formation

The high incidence of various enteric viruses in a school for mentally retarded children is comparable to that obtaining generally among young children living under poor conditions of sanitation and hygiene—during the summer months in temperate climates and at all times in subtropical and tropical climates The high incidence of enteric viruses which Ramos Alvarez and I found in a study of rectal swabs from approximately 1,800 children in Mexico in 1954, would lead one to expect very frequent interference with the usual pattern of multiplication of the orally administered poliovirus vaccine strains This was apparent in the first study reported by Ramos Alvarez on young children in Mexican nurseries, many of whom did not develop antibody until 2 to 3 months after the feeding of the vaccine strains, and a large proportion remained without demonstrable Type 3 antibody. Let me remind you that in children from 1 to 2 years of age in Veracruz, Mexico, at any one time a single rectal swab yielded a 12 per cent incidence of polioviruses All kinds of "wild" polioviruses (George Dick)—not just "mild" ones were found in monkey tests on these strains, as I have already reported in 1957. That means that if you feed vaccine in such an area to children of that age, 12 out of a 100 at any one moment may be expected to have polioviruses of their own Just prior to the current large-scale trials in Mexico, Ramos Alvarez selected 12 children without antibody for a study to be carried out in my laboratory on the presence of virus in pharyngeal and rectal swabs following the oral administration of 10 times the usual dose of the poliovirus vaccine strains (Slide 23). Multiplication of the vaccine strains of sufficient extent to be detected in tests on rectal swabs occurred in only 4 of the 12 children. Among the 8 without demonstrable multiplication, 7 yielded other enteroviruses in tissue cultures of the rectal swabs taken just before feeding of the vaccine, and one developed clinical measles 2 days after the vaccine It is noteworthy that 3 of the 7 enteroviruses that were recovered immediately before feeding of the vaccine were polioviruses (one Type 1 and two Type 2), and the others were identified as Coxsackie B-1 and B-2, ECHO 3, and a new type of enterovirus that can be recovered in human type cells but not in monkey kidney cells

Unfortunately, Dr Ramos Alvarez was able to get subsequent specimens from only four of these children, and in three of them it was evident that not only was no virus detected in the rectal

SLIDE 23

INTERFERING VIRUSES ENCOUNTERED IN A GROUP OF MEXICAN CHILDREN WITHOUT HOMOTYPIC ANTIBODY JUST BEFORE FEEDING OF LIVE POLIOVIRUS VACCINE

Type Fed 0 1 ml undiluted	Child No	Age Yrs	Age Mos	Virus Isolated from Rectal Swabs in Monkey Kidney: Just before feeding	During subsequent 3 weeks
1	1	3	10	0	Polio 1 from 4 - 21 days
	2	2	11	0	None - clinical measles at 2 days
	3	2	2	Polio 2 + N P	Other viruses for 9 days, then negative
	4	1	9	ECHO 3	ECHO 3 for 6 days, then negative
	5	1	6	Coxsackie B 2	Coxsackie B 2 for 9 days then negative
2	6	3	11	0	Polio 2 from 2 to 21 days
	7	3	7	0	Polio 2 from 4 to 9 days, then negative
	8	1	9	Polio 1	Polio 1 for 9 days then negative
	9	1	8	Coxsackie B 3	Coxsackie B 3 for 2 days then negative
	10	1	1	0 New type of enterovirus in ERK	Negative in monkey kidney 2 to 21 days Positive in "ERK" for 9 days
3	11	3	10	0	Polio 3 from 6 to 13 days
	12	3	10	Polio 2	Polio 2 at 2 days, then negative

swabs, but they also did not develop antibody at 21 to 30 days

So there was real interference.

It is obvious from these observations and from others to be reported here by Melnick and Ramos Alvarez that the high incidence of naturally occurring enteric viruses among children living under poor conditions of sanitation and hygiene, especially in subtropical and tropical climates, presents a special problem in the successful utilization of live poliovirus vaccine under these conditions. Without natural spread of the vaccine strains, the rate of effective immunization with such a vaccine would be very low. Even with natural spread, a single feeding of such a vaccine may not be sufficient for mass immunization unless massive use of the poliovirus vaccine strains will also temporarily suppress the dissemination of the other enteric viruses—a possibility that is suggested by Hale's studies in Singapore following administration of the Type 2 poliovirus vaccine to 200,000 children under 10 years of age—approximately half the child population of this age in Singapore. It is also clear, however, from the results to be reported by the investigators in the U.S.S.R. and Czechoslovakia, where the large field trials were carried out during the winter months, that interference by other enteric viruses played a minor, if any role, with approximately 97 to 100 per cent of the children developing antibody for the important Type I poliovirus

At the end of 1956, with the cooperation of the Merck, Sharp and Dohme Research Laboratories, I prepared 22 to 25 liter lots of each strain of the 3 types of attenuated poliovirus finally selected for study in human beings on a progressively larger scale—this quantity was sufficient for more than two million doses, and portions of these same original lots have been used in all the small- and large-scale studies The results of the original basic studies in the United States have been published Prior to using these lots in the large field trials, basic studies involving groups of hundreds to thousands of persons were carried out by Prof. J D Verlinde and his associates in Holland, by Doctor M. Ramos Alvarez and Prof. F. Gómez in Mexico City, and by Prof A. A Smorodintsev and his associates in Leningrad These investigators

have already reported the results of their extensive studies Portions of these same lots were also used for basic studies on family groups in the United States by Doctors John P. Fox and Henry M Gelfand and their associates of Tulane University, and by Doctors John R. Paul and Dorothy M. Horstmann and their associates of Yale University The first small field trial with all 3 types of the vaccine, involving approximately 3,000 children in Mexico City, was carried out by Ramos Alvarez and Gómez during the first half of 1958. Professor James H. Hale, in cooperation with the Ministry of Health in Singapore, carried out the first large-scale field trial, using the Type 2 vaccine on opproximately 200,000 children from 3 months to 10 years of age, in an attempt to modify a rapidly developing epidemic of paralytic poliomyelitis caused by the Type 1 virus—and he is reporting the results of this interesting study at this meeting Professors Smorodintsev and Chumakov received approximately 225,000 doses of aliquots of each of the three lots and they began their field trials on progressively larger numbers of children in the U S S.R late in 1958 They used a portion of each of these lots to prepare secondary lots of vaccine in their own laboratories, and by the end of May 1959, a total of about 3,860 000 persons in various republics of the U.S S R had received one or more types of either the original or the secondary lots of this vaccine In Czechoslovakia Doctors V Skovranek and K. Zacek and their associates of the Ministry of Health began a large field trial and extensive virologic and epidemiologic study of the effect of administering the 3 types of live poliovirus vaccine to children 2 to 8 years of age, who had previously had 3 doses of Salk vaccine, the last dose having been given 8 months earlier. However, serologic surveys carried out shortly before the feeding of the live vaccine indicated that of the 143,377 children who received the Type 1 vaccine, an estimated 48,000 had no demonstrable Type 1 antibody, of the 127,290 children, who then received the Type 3 vaccine, an estimated 55,000 had no demonstrable Type 3 antibody, and of the 114,510 children who received the Type 2 vaccine, an estimated 23,000 had no demonstrable Type 2 antibody. The large field trials in Mexico began at the end of February 1959, and Dr. Ramos Alvarez informed me that by this time approximately 170,000 or more children have received one or more types—all from the orginal lots of vaccine By this time approximately 4 5 million persons in different parts of the world had received one or more types—either from the original lots that I had prepared or from the secondary lots that were prepared in the U.S S R The available results on these extensive studies will be presented at this conference by the responsible investigators Last month I had an opportunity to study the results during visits to Prague, Moscow and Leningrad, and also to read Dr. Hale's report on his study in Singapore, and I concur in the conclusion of these investigators that the vaccine had proved to be entirely safe and of high immunogenic potency

DISCUSSION

Chairman Anderson: The paper presented by Dr Sabin is now open for discussion. May we remind you to please limit yourselves to the actual material presented Any discussion of the field trials will be deferred until later

Dr Gear I would like to ask Dr Sabin whether he has considered a problem we recently faced He mentioned the difficulties created in his live vaccination program by the infection with other enteroviruses

In September of last year, we had arranged to feed with live poliovirus vaccine the small children and infants in an institution One week before this administration was to be given, I received a telephone call from Dr Peacock, the medical officer concerned, who informed me that there had been three cases of poliomyelitis in this institution, two of them non-paralytic and one fatal

I was of course greatly relieved that this had happened one week before and not one week after the time of the administration of the vaccine. However, subsequent investigation showed that this outbreak was caused by Coxsackie A-2 virus and not by poliovirus.

My question concerns the possibility of a synergism between Coxsackie A viruses and the avirulent polioviruses It is one of the problems to which we must have an answer before lightly undertaking the feeding of live poliovirus vaccine. If there is a synergic action, as the work of Dr. Dalldorf suggests, obviously the administration of live poliovirus may be more dangerous under those circumstances than when the children are not suffering from a Coxsackie A virus infection.

Dr. Sabin: I think that the point raised by Dr Gear has come up repeatedly during recent months in discussions based on Dr. Dalldorf's article in the *Journal of Experimental Medicine*. I think it would be important, very important for us, to understand the problem clearly. I regret to say that it was not clearly presented in the original article.

Dr Dalldorf gave me a very specific prescription for a partly attenuated strain of virus that he wanted. He did not want any of the vaccine strains He wanted a strain of virus which, upon inoculation intracerebrally, would destroy enough anterior horn cells not to produce paralysis by itself, at least not often. I sent him such a strain, isolated from a child with minor illness

He inoculated this strain intracerebrally and several days followed it with an intracerebral inoculation of special mutant of Coxsackie A-14, which, by itself, after inoculation intracerebrally, can also destroy a further number of anterior horn cells And so, the combination has produced the paralysis that each alone would not do

The question is, what are the possibilities of such a thing occurring under natural conditions? If one were dealing with a virus which invaded the nervous system, i e, if the vaccine strains invaded the nervous system, and their failure to produce an effect depended entirely on their inability to produce change in a large enough number of neurons, then one might visualize the possibility that if such a naturally occurring neurovirulent Coxsackie virus would invade at the appropriate time—then, perhaps, a combined effect might add up to a worse situation

But with the vaccine viruses that I am using there is no evidence that they can invade the central nervous system; their activity depends upon multiplication in the intestinal tract without invasion

Furthermore, I do not know of any instance in which both Coxsackie and poliomyelitis virus have been shown to be the cause of the natural disease—i e, evidence that both actually multiplied in the nervous system At the present time, all the evidence we are getting is that the Coxsackie viruses are much more likely to interfere than to act in a synergistic manner in the nervous system

Chairman Anderson: Are there any other questions or comments? Does that answer your question, Dr. Gear?

Dr. Gear: Yes

DR SABIN: I would like to ask Dr. Gear a question, if I may, Mr. Chairman. What was the pathology in the fatal case that was attributed to infection with Coxsackie A 2? If we are going to think clearly about this, let us not merely have a hearsay statement suggesting that Coxsackie A 2 was the cause of the fatal paralytic disease.

DR GEAR: There was no postmortem done on this particular case, but since then we have had two other fatal cases apparently due to Coxsackie A virus, because the virus was isolated postmortem from the tissues, from the heart muscle, from the brain, and from the blood as well as from the bowel Therefore, there is good evidence that the Coxsackie A virus was the cause of death No other virus was isolated. There were, of course, large numbers of postmortem bacteria

DR. SABIN What was the pathology?

DR GEAR: The pathology is under study, and I will let Dr Sabin know as soon as we have it

We have had another case, a girl aged 17, clinically a fatal case of poliomyelitis, from whose central nervous system both poliovirus, Type 1, and Coxsackie A virus, Type 4, were isolated These postmortem isolations have, however, raised in our mind the possibility that the virus may be taken into the tissues by the agonal or postmortem bacterial invasion But that is a question which we cannot answer, and I presume it cannot yet be answered

CHAIRMAN ANDERSON: May I suggest, if you furnish that information to Dr Sabin, that it be furnished also to the Conference, since the question which has been raised will appear in the Conference recordings

DR GEAR Certainly, Mr Chairman

DR SABIN I do not wish to press Dr Gear too hard, but I think it is important for us to know whether death under those circumstances was due to involvement of anterior horn cells or whether it was due to changes in the choroid plexus and other areas Nor do I think it is so impossible to determine whether the isolation of two viruses or any virus from the central nervous system is an agonal affair or whether it actually had undergone multiplication

I think that instead of referring merely to isolations of virus, it would be extremely helpful if quantitative studies would be carried out

We know the difficulty with a case that was brought up some years ago by Dr. Steigman, where only a trace of Coxsackie B-2 was present in the spinal cord of a child who died with paralytic polio. Can we under such circumstances merely assume that this was the virus that had been multiplying? There are difficulties, but quantitative studies should help a great deal

CHAIRMAN ANDERSON. Dr. Dick

DR DICK: May I ask one question Does viremia ever occur with your present optimal strains?

DR. SABIN: With Type 1 and Type 3 repeated tests have failed to show this With Type 2, the findings of a trace of virus in 2 cases were reported by me in 1957 and I am sure Dr Dick knew the answer to that beforehand But I would like again to ask Dr Dick at all times to speak and think quantitatively, rather than qualitatively; not to speak of viremia, but of how much viremia Studies by Dr Bodian had shown very clearly that just a trace of virus in the blood stream has one significance; the presence of a great deal has another significance So that when we say that none was detected, it is possible that it may have been below the threshold of detection, and that in the two instances in which it was detected perhaps just the peak had been found

DR. DICK The only reason I asked that question is that if there is viremia you cannot really say that virus may not get to the central nervous system

DR. SABIN. The mere presence of a small amount of virus in the blood stream, as Dr Bodian has shown, is not conducive to its getting to the nervous system by whatever method may occur, and intraspinal inoculations in chimpanzees have shown the innocuity of large doses that are put in artificially.

Dr. Bell: I would like to ask Dr Sabin if he noted any evidence of illness after artificial feeding of virus. In a study of the natural occurrence of polio infections among children in an institution, we found that a mild illness occurred at the time of initial infection with polio Type 3 or Type 2 virus A mild febrile illness occurred within two or three days of infection, and infection occurred within two or three days after exposure. I wondered if children fed attenuated viruses were carefully checked, with temperatures, to see whether any illness occurred.

I also have a comment This interference phenomenon is extremely interesting to us because in our studies of institutionalized children, 6 to 35 months of age, we have observed repeated outbreaks of virus infections Each child gets a new virus infection on an average of about once every four weeks

An average of forty per cent of the children are shedding a virus all the time These infections occur in sharp outbreaks ECHO 8, ECHO 13, ECHO 12, and ECHO 14 occurred successively one right after the other, with only two or three weeks' intervals between each outbreak It has been difficult to interpret the data. I feel that interference is involved, and your data give me confidence in that deduction

However, it is important to note that some outbreaks occur simultaneously. For example, Coxsackie Type B-5 occurred simultaneously with ECHO 7, and ECHO 14 occurred simultaneously with polio 2 infection There may be interference with some virus types but with others there may not be interference at all

Dr. Sabin: The first attempt to answer this question was carried out with young adults living in an isolated institution and, as I previously reported with earlier strains derived from healthy children, there were some instances in which, three to four days after feeding, there was no fever but pharyngitis and occasionally abdominal pain, which led me to believe that these manifestations were associated with infection

When I undertook the study with the current vaccine strain on my own two triple-negative children and their three playmates, also triple-negatives, I thought: "I am going to do this very carefully now," and, like Dr. Gear, I set up certain time schedules. I said: "I am going to start to give the vaccine now." Every time I said "I am going to start to give it" and did not give it, two to three or four days later they came down with either pharyngitis, vomiting and abdominal pain, or a little fever.

I waited for approximately six weeks for those children to stop having some sort of febrile episode I finally gave up It so happened that after they got the vaccine they did not have any such episode

However, a report later to be given by Dr Smorodintsev will deal with approximately 7,500 children who had received the vaccine and were carefully followed, as compared with another group, in similar number, who had not, for various types of illnesses which were occurring during the period. He will report on that study, which showed that no illness was attributable to the vaccine

I think at the present time it is very difficult to say definitely that there is not an occasional pharyngitis or minor illness, but it is also very difficult to say that there is. At least, there is not such an increase that it becomes obvious

Dr Rhodes: Since we are dealing with general questions, would Dr Sabin be so kind as to explain how he thinks the antibody is developed in people who have been fed live viruses What is the exact mechanism of pathogenesis, if we might use that term?

Dr. Sabin The basis for my answer will be observations in chimpanzees that have been fed these strains and then sacrified and their various tissues studied. These observations showed that, in the absence of demonstrable viremia, the regional lymph nodes invariably are invaded, and the amount of virus in the regional lymph nodes is proportional to that found in the portion of the alimentary tract which they drained. Thus, in the chimpanzee it was very high in the cervical lymph nodes, and low in the mesenteric lymph nodes.

It would appear that the regional lymph nodes of the alimentary tract are quite as capable of acting as antibody-forming sites as lymph nodes elsewhere in the body. As for the idea that you must have a viremia in order to have antibody formation, or, as Dr. Dick intimated, that the persistence of antibody may be longer when there is viremia than when there is no viremia—

I personally know of no evidence for it and much evidence against it

I would like to mention here another point that has been raised by Sir MacFarlane Burnet in an attempt to explain the difference between the resistance of the intestinal tract after natural and experimental infections, and the absence of resistance in those who developed antibody followed inoculation of killed vaccine. He suggested that perhaps the regional lymph nodes which are known constantly to shed lymph cells into the alimentary tract, may be affected after natural infection, but not after inoculation of killed virus vaccine

He also suggested that this constant release of antibody producing lymphoid cells from the regional lymph nodes may provide antibody in the intestinal tract and be the basis of the local immunity As a result of this suggestion we have, during the past year, carried out an extensive study in our laboratory on the stools of children and adults with susceptible and non-susceptible intestinal tracts, before and after feeding of vaccine, and were able to find absolutely no evidence that resistance of the intestinal tract had any connection with the presence or absence of antiviral bodies in the stools If the lymphocytes do get into the stools and provide antibody, it is not detectable by the sensitive methods that we used

DR BODIAN I would like to pursue the point which Dr Dick raised in relation to viremia, and to ask Dr Sabin whether he has conducted studies on those children who excreted virus more virulent than that which was fed Were these children studied for evidence of viremia at the time when more virulent mutants were present in the feces? In other words, we not only have the problem of the frequency of occurrence of viremia in those who were fed the vaccine strains, but also the problem in contacts excreting more virulent material

DR SABIN In the first place, the viremia studies that were made were carried out on individuals in whose stools a modification of the virus could be demonstrated as well as in those in whom this could not be demonstrated

In no instance was viremia demonstrable in individuals whose stools later yielded virus of greater neurotropism As a matter of fact, the very time when a modification of the excreted virus may be detected is the time when the individual already has antibody, because this phenomenon is encountered late rather than early In the early stages, before antibody develops, there has apparently not yet been sufficient multiplication to provide some virus with altered properties.

In speaking of the character of the virus in the stools, I wish to stress once again that we must think quantitatively. We must think of the ratio between changed and unchanged particles in the population of virus particles in the stools, and also of the changes which are not present in the stools but occur after the virus is inoculated into the monkey, because continued multiplication of the virus in the alimentary tract results in a loss rather than in an increase of such changed virus Studies that I have carried out on cultures grown from terminal dilutions of stools have, for example, yielded different results from cultures grown from the undiluted stool extracts upon inoculation in monkeys

DR. BODIAN I would merely like to add that I believe Dr Sabin is minimizing this problem which is admittedly very difficult In chimpanzees we have found that viremia of short duration, and not very high titer, is associated with subsequent paralytic disease

And I confess that I am not clear yet as to the role of viremia in relation to the live virus vaccine, but I do not think this problem can be brushed aside It is something we simply do not have enough information about A chimpanzee bled on one day will have a moderate quantity of virus in the serum On the next day the virus will be absent The persistence of high levels, such as we found in cynomolgus monkeys, may not be essential to invasion of the central nervous system

DR SABIN: Let us agree, at least, that things are not being brushed aside Let us say that we might disagree on the extent to which certain things have received study But I hope that Dr Bodian realizes that nobody is brushing things aside I would not have taken the trouble of spending several months studying viremia with different strains in chimpanzees and human volunteers, and viremia produced by certain low temperature mutants to correlate it with their

invasive capacity, if I were merely brushing it aside

CHAIRMAN ANDERSON. We will agree that there is no brushing aside, but at least there are certain uncertainties Dr Melnick

DR MELNICK: Dr. Sabin has raised the question of neurotropic viruses being artificially selected in the course of their isolation, and this we will discuss at greater length tomorrow I would just like to indicate here that it is possible to obtain sufficient virus for monkey tests in the rectal swabs from persons who have been fed with the vaccine strains, and that this virus may already be pathogenic for monkeys when properly inoculated I say "properly" in quotes, because to Dr. Sabin properly inoculated means one thing, and to me it means another Specifically, when such rectal swabings are inoculated intraspinally, with a fine needle, they may produce paralysis in monkeys which can be confirmed histologically as due to poliovirus multiplication

DR SABIN: May I comment on this? I am glad Dr Melnick finally said "inoculated intraspinally," and put it in that way. The original virus, when inoculated this way in a slightly larger amount, will also produce a similar effect, because the virus can multiply. So that, in addition to further selection that you can get in a monkey, this is a process by which the virus is distributed over a large area in the lumbar cord, and by itself is no different from what has been reported before, namely, that the material, as it occurs in the stools, in the quantities that it occurs when inoculated intracerebrally, does not have such an effect.

CHAIRMAN ANDERSON: If there are no further questions, I think we can close the discussion at this point

2. COMPARATIVE VIRULENCE FOR RHESUS MONKEYS OF POLIOVIRUS STRAINS USED FOR ORAL ADMINISTRATION

R. Murray, R. Kirschstein, G. Van Hoosier, Jr., and S. Baron

Division of Biologics Standards,
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Dr Murray *(presenting the paper)*: Comparative data are presented showing the degree of neurovirulence for Rhesus monkeys of three Type 1, three Type 2, and three Type 3 poliovirus strains used for immunization by the oral route These studies were designed to demonstrate whether any differences exist between the various strains proposed for such use when tested by intraspinal and intracerebral inoculation of Rhesus monkeys under strictly comparable conditions in one laboratory

The studies described in this report were carried out in an effort to obtain, under standardized conditions, comparative information concerning the neurovirulence for monkeys of the various strains presently proposed for use in immunization against poliomyelitis by oral administration The proposal was to test each of the strains in groups of monkeys of the same species by intraspinal and intracerebral inoculation using identical methods and techniques both in the inoculation of the animals and in the interpretation of the results It was hoped that by this means it would be possible to obtain comparative quantitative information which would be considered along with field trial and other data which are being accumulated in the evaluation of these strains

It seemed appropriate that such studies should be conducted in our laboratories at this time since we have become familiar with the use of monkeys in the safety test for inactivated poliomyelitis vaccine over the past four years This program, which has involved some 20,000 animals, has provided invaluable experience in the handling and inoculation of these animals as well as in the interpretation of the lesions seen in histopathologic sections of the central nervous system

STRAINS STUDIED

Table 1 shows the strains which were studied. These materials were obtained directly from Dr Koprowski, Dr Cox of the Lederle Laboratories, and from Dr Sabin. The information available concerning the titers of the nine strains is also shown in Table 1.

MONKEYS

Only Rhesus monkeys (*Macaca mulatta*) were used All animals were in overt good health and weighed between 3 and 7 lbs. initially

Table 1 Strains of Poliovirus Studied

Koprowski group		
Type 1	Wistar-Chat, Pool 13	$10^{7.6}$ TCD_{50} per ml
Type 2	Wistar, Pool 1	$10^{6.2}$ TCD_{50} per ml
Type 3	Wistar-Fox, Pool 3	$10^{7.7}$ TCD_{50} per ml
Lederle group		
Type 1	Lederle-SM, #7-1231-114	$10^{6.7}$ TCD_{50} per ml
Type 2	Lederle-MEF-1, #7-1232-243	$10^{7.2}$ TCD_{50} per ml
Type 3	Lederle-Fox, #7-1233-344	$10^{7.7}$ TCD_{50} per ml
Sabin group		
Type 1	L Sc, 2 ab	$10^{7.8}$ TCD_{50} per ml
Type 2	P 712, Ch, 2 ab	$10^{7.3}$ TCD_{50} per ml
Type 3	Leon, 12 a₁b	$10^{7.3}$ TCD_{50} per ml

They were observed daily for signs of poliomyelitis and were sacrificed at 17 to 20 days During the period of the test the animals were housed in double-decked stainless steel monkey cages with two or three animals being housed per cage Animals were observed daily and scored on the basis of weakness, partial paralysis or complete paralysis of the limbs. All animals were bled prior to inoculation and at the conclusion of the test Approximately 10 per cent of the initial blood samples remain to be tested for the presence of poliovirus antibodies Of the remainder, four were positive when screened for poliomyelitis antibodies The results of second sample assays are not yet available.

INOCULATION OF MONKEYS

Monkeys were inoculated either intracerebrally or intraspinally under deep "Nembutal" anesthesia

The intracerebral injections consisted of 0 5 ml of material placed into each thalamic area Administration was made by means of a 1 0 ml. tuberculin syringe fitted with a 1¼" (3 3 cm.) 25 gauge needle The technique of locating the thalamus was that described by Bodian *et al* * All animals included in the present study were injected intrathalamically and are designated in this way in the tables

The intraspinal inoculation consisted of 0 2 ml of material injected into the lumbar enlargement This was accomplished by means of a 1 0 ml tuberculin syringe fitted with a ¾" (1 9 cm) 27 gauge needle The technique used is as follows

The needle is inserted in the space between L_1 and L_2, slightly to one side of the midline The needle is inserted until a muscular twitch is observed in one or both legs At this time the inoculation is slowly begun and, if properly located, is accompanied by convulsive movements in the muscles of one or both legs throughout the period of inoculation Frequently it is necessary to go a little deeper after the first muscular twitch is observed to get a satisfactory reaction during inoculation If difficulty is encountered at this site (L_1-L_2) an attempt is then made to inoculate in the space between T_{12} and L_1.

No cortisone or penicillin was used.

* Bodian, D., Morgan, I. M., and Schwerdt, C. E Am J. Hyg *51:* 126-133, 1950

MATERIALS FOR INOCULATION

A series of 10-fold dilutions was prepared for each of the materials to be inoculated Medium 199 was used as the diluting fluid. Five or more monkeys were used for each dilution administered intraspinally and for each dilution administered intracerebrally. Control monkeys were inoculated with Medium 199 under identical conditions Such animals, which numbered 13 in the case of those inoculated intraspinally, and 14 intracerebrally, were spaced in time to cover the entire test period in order to study the effect of inoculation trauma and to control the possibility of cross-infection. None of the controls showed either progressive paralysis or lesions of poliomyelitis

EXAMINATION OF TISSUES

At the end of the observation period of 17 to 20 days the animals were sacrificed Sections of lumbar cord, cervical cord and frontal cortex were removed and stored frozen at −20°C for virus isolation studies. The remainder of the CNS tissue was preserved in "formalin" for histologic examination The methods followed were those used in the monkey safety test for inactivated poliomyelitis vaccine.*

Histopathologic examinations of the CNS tissues were made as follows

(1) Three blocks of the cervical enlargement C_6 to T_1 Blocks were split and six sections examined

(2) Three blocks of the lumbar enlargement L_4 to S_1. Blocks were split and six sections examined

(3) Three blocks of thoracic cord

(4) Sections of the lower medulla, the upper medulla and the mesencephalon at the level of the inferior colliculus

Sections 15μ in thickness were cut and stained with gallocyanin Each section was evaluated individually for lesions, which were graded as minimal, mild, moderately severe, and severe In addition, each section of the lumbar cord of intraspinally inoculated animals was evaluated for the presence or absence of inoculation trauma and the brains of intracerebrally inocu

* Technical Committee on Poliomyelitis Vaccine and Subcommittee on the Monkey Safety Test, Am J Hyg. *64:* 104-137, 1956

lated animals were examined grossly as to location of the inoculation trauma

DETERMINATION OF VIRUS TITERS

Serial tenfold dilutions of virus were made in Medium 199 or Eagle's basal medium (BME) and assayed by the plaque method using Medium 199 containing 20 per cent skim milk* as the overlay medium Titers were expressed as numbers of plaque forming units (PFU) per ml Some additional assays were done by the tube titration method using four washed tubes containing 1.0 ml. BME per tenfold dilution Fifty per cent tissue culture infectious doses ($TCID_{50}$) were calculated by the method of Reed and Muench

VIRUS ISOLATION STUDIES

The present program provided an opportunity for studying the spread of viruses within the central nervous system of the inoculated monkeys and of examining the characteristics of the recovered strains. In addition, serial rectal swabs were taken from representative monkeys prior to inoculation, on selected days throughout the observation period, and at the time of autopsy These swabs were taken in order to determine whether poliovirus was being shed with the feces during the observation period—a circumstance which might have reflected on the validity of the basic purpose of this comparative study Approximately 50 per cent of this work has been completed, and although numerous simian agents have been isolated from the stools, no poliovirus has yet been found The methods used in the virus isolation studies were as follows

Specimens for virus isolation. Specimens of lumbar cord, cervical cord, frontal cortex or rectal swabs were each shaken with glass beads in a conical centrifuge tube containing BME 1,000 units of penicillin, 1,000 µg streptomycin and 500 µg "Mycostatin" per ml to make a 20 per cent suspension

Cell culture Rhesus monkey kidney cell cultures were propagated in Medium 199 containing 2 per cent calf serum, 100 units of penicillin and 100 µg streptomycin After cell sheet formation and just prior to inoculation of specimens, culture tubes were rinsed three times with 1 ml Earle's balanced salt solution (BSS), fed 1 ml. BME containing 100 units of penicillin and 100 µg streptomycin per ml.

Virus isolation from CNS specimens One-tenth ml of each specimen was inoculated into each of two tube cultures, placed at 36°C. and observed for seven days One-tenth ml of fluid from tubes manifesting cell degeneration was transferred to fresh culture tubes to confirm the presence of a transmissible agent Cultures showing cell degeneration on subculture were stored at -20°C. until virus identification could be performed

Virus isolation from stool specimens These specimens were handled in the same manner as the CNS specimens except that specimens contaminated with bacteria or molds were filtered through Swinney filters previously treated with 1 ml of tryptose phosphate broth to allow passage of poliovirus through the filters Fluids from subcultures showing degeneration were assayed by plaque formation Poliovirus identification tests were performed only on those agents capable of forming plaques

RESULTS

VIRUS TITERS: The results of the titers obtained for each of the materials studied were determined as $TCID_{50}$ and in terms of PFU per ml These results are shown in Table 2 in comparison with the results reported to us It will be noted that differences are not great In some cases repeat determinations have been made since this table was prepared, these substantiate the values shown The titers reported under the PFU column will be used in the subsequent tables and discussions of the titers of the material studied

In view of the fact that there were some differences between the initial titers of the individual strains studied an attempt was made to adjust the dilutions at which the end points occurred by noting the number of PFU/ml, which could be administered without resulting in the production of lesions or of weakness or paralysis as the case may be These figures are referred to in the tables as the "thresholds" The figures were obtained by subtracting the exponent to base 10 of the first dilution at which no positive findings were obtained from the initial titer In this scheme, therefore, the

* Baron, S., and Low, R J: Science, 128: 89 90, 1958

They were observed daily for signs of poliomyelitis and were sacrificed at 17 to 20 days During the period of the test the animals were housed in double-decked stainless steel monkey cages with two or three animals being housed per cage Animals were observed daily and scored on the basis of weakness, partial paralysis or complete paralysis of the limbs All animals were bled prior to inoculation and at the conclusion of the test Approximately 10 per cent of the initial blood samples remain to be tested for the presence of poliovirus antibodies Of the remainder, four were positive when screened for poliomyelitis antibodies. The results of second sample assays are not yet available

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(2) Three blocks of the lumbar enlargement L_3 to S_1 Blocks were split and six sections examined

(3) Three blocks of thoracic cord

(4) Sections of the lower medulla, the upper medulla and the mesencephalon at the level of the inferior colliculus

Sections 15μ in thickness were cut and stained with gallocyanin Each section was evaluated individually for lesions, which were graded as minimal, mild, moderately severe, and severe In addition, each section of the lumbar cord of intraspinally inoculated animals was evaluated for the presence or absence of inoculation trauma and the brains of intracerebrally inocu-

* Technical Committee on Poliomyelitis Vaccine and Subcommittee on the Monkey Safety Test· Am J. Hyg *61:* 104-137, 1956

TABLE 3B COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 1—INTRATHALAMIC INOCULATION

Diln	KOPROWSKI TITER-7 6 PFU/1 ML			LEDERLE TITER-6 4 PFU/1 ML			SABIN TITER-7 4 PFU/1 ML		
	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM
10^{0}	2/5*	2/5	2/5	1/5	2/5	2/5	0/9	0/9	0/9
10^{-1}	0/5	0/5	2/5	2/5	2/5	3/5	0/4	0/4	0/4
10^{-2}	2/5	2/5	2/5	3/5	1/5	3/5	0/5	0/5	0/5
10^{-3}	2/5	2/5	2/5	1/5	2/5	1/5	0/5	0/5	0/5
10^{-4}	0/5	0/5	0/5	1/5	1/5	1/5	0/5	0/5	0/5
10^{-5}	0/5	0/5	0/5	—	—	—	—	—	—
Threshold PFU as Log_{10}	3 6	3 6	3 6	<2 4	<2 4	<2 4	>7 4	>7 4	>7 4

* No of monkeys showing lesions of polio/No of monkeys inoculated and surviving the test period.

TABLE 4A COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 2—INTRASPINAL INOCULATION

Diln	KOPROWSKI TITER-4 9 PFU/1 ML			LEDERLE TITER-6 5 PFU/1 ML			SABIN TITER-7 4 PFU/1 ML		
	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM
10^{0}	—	—	—	4/4*	4/4	4/4	—	—	—
10^{-1}	4/4	2/4	3/4	5/5	5/5	5/5	—	—	—
10^{-2}	0/4	0/4	0/4	5/5	5/5	5/5	5/5	0/5	0/5
10^{-3}	0/5	0/5	0/5	4/5	4/5	4/5	1/4	0/4	0/4
10^{-4}	0/4	0/4	0/4	3/5	3/5	3/5	2/5	0/5	0/5
10^{-5}	—	—	—	0/5	0/5	0/5	0/5	0/5	0/5
Threshold PFU as Log_{10}	2 9	2 9	2 9	1 5	1 5	1 5	2 4	>5 4	>5 4

* No of monkeys showing lesions of polio/No of monkeys inoculated and surviving the test period

TABLE 2 COMPARATIVE TITRATION OF ATTENUATED POLIOVIRUSES

Titer Reported By Source			D.B.S. Results	
Source	Virus Type	$TCID_{50}$	$TCID_{50}$	PFU
Koprowski	1	7 5	7 2	7 6
Koprowski	2	5 2	5 5	4 9
Koprowski	3	7 7	7 4	7 5
Sabin	1	7 8–8 1	7 2	7 4
Sabin	2	7 3	8 0	7 4
Sabin	3	7 2–7 4	6 2	7 3
Lederle	1	6 8	6 4	6 4
Lederle	2	7 1	6 2	6 5
Lederle	3	7 7	7 0	7 3

TABLE 3A COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 1—INTRASPINAL INOCULATION

Diln	KOPROWSKI Titer-7 6 PFU/1 ml †			LEDERLE Titer-6 4 PFU/1 ml †			SABIN Titer-7 4 PFU/1 ml †		
	Lumbar cord	Cervical cord	Brain stem	Lumbar cord	Cervical cord	Brain stem	Lumbar cord	Cervical cord	Brain stem
10^{0}	5/5*	5/5	4/5	5/5	5/5	5/5	5/5	4/5	3/5
10^{-1}	5/5	4/5	5/5	5/5	3/5	4/5	5/5	2/5	2/5
10^{-2}	5/5	4/5	5/5	5/5	4/5	4/5	5/5	3/5	4/5
10^{-3}	4/5	3/5	4/5	5/5	3/5	5/5	5/5	2/5	3/5
10^{-4}	4/5	4/5	4/5	4/5	3/5	3/5	2/5	1/5	1/5
10^{-5}	0/5	0/5	0/5	0/5	0/5	0/5	0/4	0/4	0/4
Threshold PFU as Log_{10}	2 6	2 6	2 6	1 4	1 4	1 4	2 4	2 4	2 4

* No of monkeys showing lesions of polio/No of monkeys inoculated and surviving the test period
† Initial titer—7 6 plaque forming units (PFU)/ml

TABLE 5B COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 3—INTRATHALAMIC INOCULATION

Diln	KOPROWSKI Titer-7.5 PFU/1 ML			LEDERLE Titer-7 3 PFU/1 ML			SABIN Titer-7 3 PFU/1 ML		
	Lumbar cord	Cervical cord	Brain stem	Lumbar cord	Cervical cord	Brain stem	Lumbar cord	Cervical cord	Brain stem
10^0	1/5*	2/5	3/5	1/5	2/5	2/5	0/10	0/10	0/10
10^{-1}	4/5	4/5	4/5	1/5	1/5	1/5	0/4	0/4	0/4
10^{-2}	1/5	1/5	1/5	1/5	1/5	1/5	0/5	0/5	0/5
10^{-3}	2/5	2/5	2/5	0/3	0/3	0/3	0/5	0/5	0/5
10^{-4}	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10^{-5}	0/5	0/5	0/5	—	—	—	—	—	—
Threshold PFU as Log_{10}	3 5	3 5	3 5	4 3	4 3	4 3	>7 3	>7 3	>7 3

* No of monkeys showing lesions of polio/No of monkeys inoculated and surviving the test period.

threshold value would be inversely related to the degree of neurotropism

HISTOLOGIC FINDINGS The results obtained are shown in Tables 3a, 4a, and 5a in the case of animals *injected by the intraspinal route* and in Tables 3b, 4b, and 5b in the case of those injected by the intrathalamic route Animals which failed to exhibit direct gross or histologic evidence of inoculation trauma in the thalamus or in the lumbar spinal cord, respectively, were excluded from the study The tables are self explanatory and require little discussion

a) *Intraspinal inoculation* It is immediately apparent that all of the strains tested by intraspinal inoculation were neurotropic, but there appeared to be differences of degree *Type 1* Lesions were found through the 10^{-4} dilutions for each of the three strains It may be noted that the Lederle Type 1 had a titer of 6 4 PFU/1 ml as compared with 7 6 for the Koprowski strain and 7 4 for the Sabin strain *Type 2* There are more obvious differences than was the case with Type 1 *It is difficult to evaluate the Koprowski* Type 2 because of its initial low titer While both the Lederle and Sabin strains showed lesions through the 10^{-4} dilution, there was less tendency for the lesions to be seen in the cervical and brain stem sections in the case of the latter It may be noted that the titer of the Lederle strain was 6 5 vs 7 4 for the Sabin strain *Type 3* The titers were comparable for all three strains Lesions were seen in sections from animals injected with 10^{-5} dilutions in the case of both the Koprowski and Lederle materials. With the Sabin material, lesions were seen only through the 10^{-4} dilution *and here again, as with* the Sabin Type 2 strain, there was less tendency for lesions to be seen in the cervical and brain stem sections

b) *Intrathalamic inoculation* In viewing the tables it is immediately apparent that the picture presented is quite different from that *seen in the* case of intraspinally inoculated animals. With some strains no lesions were found in the lumbar, cervical, or brain stem sections at any dilution, whereas with others, lesions were seen even with high dilutions No lesions were seen at any of the dilutions tested in the case of the Sabin strains. With the Koprowski strains lesions were seen through 10^{-3} for Type 1 and through 10^{-3}

TABLE 4B COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 2—INTRATHALAMIC INOCULATION

Diln	KOPROWSKI TITER-4 9 PFU/1 ML			LEDERLE TITER-6 5 PFU/1 ML			SABIN TITER-7 4 PFU/1 ML		
	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM
10^{0}	0/5*	0/5	0/5	4/5	4/5	4/5	0/4	0/4	0/4
10^{-1}	0/5	0/5	0/5	4/5	4/5	4/5	0/5	0/5	0/5
10^{-2}	0/5	0/5	0/5	5/5	5/5	5/5	0/5	0/5	0/5
10^{-3}	0/5	0/5	0/5	4/5	4/5	4/5	0/4	0/4	0/4
10^{-4}	0/4	0/4	0/4	3/5	3/5	3/5	0/4	0/4	0/4
10^{-5}	—	—	—	1/5	1/5	1/5	—	—	—
Threshold PFU as Log_{10}	>4 9	>4 9	>4 9	<1 5	<1 5	<1 5	>7 4	>7 4	>7 4

* No of monkeys showing lesions of polio/No of monkeys inoculated and surviving the test period

TABLE 5A COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 3—INTRASPINAL INOCULATION

Diln	KOPROWSKI TITER-7 5 PFU/1 ML			LEDERLE TITER-7 3 PFU/1 ML			SABIN TITER-7 3 PFU/1 ML		
	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM
10^{0}	5/5*	3/5	2/5	5/5	4/5	4/5	—	—	—
10^{-1}	4/4	3/4	3/4	5/5	4/5	5/5	5/5	2/5	2/5
10^{-2}	5/5	1/5	3/5	5/5	5/5	5/5	5/5	2/5	1/5
10^{-3}	5/5	1/5	3/5	4/4	1/4	0/4	3/5	0/5	0/5
10^{-4}	0/5	0/5	0/5	2/5	1/5	2/5	2/4	0/4	0/4
10^{-5}	1/5	1/5	1/5	2/5	2/5	2/5	0/4	0/4	0/4
Threshold PFU as Log_{10}	<2 5	<2 5	<2 5	<2 3	<2 3	<2 3	2 3	4 3	4 3

* No. of monkeys showing lesions of polio/No. of monkeys inoculated and surviving the test period

TABLE 7 SUMMARY OF COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS TYPE 2

		KOPROWSKI		LEDERLE		SABIN	
ROUTE	DILN	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS
INTRASPINAL	10^{0}	—	—	4/4	3/4	—	—
	10^{-1}	4/4*	3/3	5/5	3/5	—	—
	10^{-2}	0/4	0/4	5/5	5/5	5/5	3/5
	10^{-3}	0/5	0/5	4/5	3/5	4/4	0/4
	10^{-4}	0/4	0/4	3/5	2/5	2/3	0/3
	10^{-5}	—	—	0/5	0/5	0/5	0/5
	Threshold PFU/ml	2.9	2.9	1.5	1.5	2.4	4.4
INTRATHALAMIC	10^{0}	0/5	0/5	4/5	0/5	0/4	0/4
	10^{-1}	0/5	0/5	4/5	0/5	0/5	0/5
	10^{-2}	0/5	0/5	5/5	0/5	0/5	0/5
	10^{-3}	0/5	0/5	4/5	0/5	0/4	0/4
	10^{-4}	0/4	0/4	3/5	1/5	0/4	0/4
	10^{-5}	—	—	1/5	0/5	—	—
	Threshold PFU/ml	4.0	4.0	1.5	1.5	7.4	7.4

* No with findings/No inoculated

† Only definite progressive weakness or paralysis beyond day 2 scored as positive

paralysis; in the case of the latter no more than weakness of one arm and leg was observed These effects were observed one to two days following inoculation Only when there was definite *progression of weakness* or *paralysis after the* second day were the animals considered positive Questionable findings were considered negative

Tables 6, 7, and 8 give a comparison between the histopathologic findings and the number of animals showing weakness and paralysis

a) *Intraspinal inoculation* *Type 1* Examples of the scoring of inoculated animals are shown in Tables 9, 10, and 11 There was close correlation between progressive weakness and paralysis and the histopathologic findings for both the Koprowski and Lederle strains With the Sabin strain histopathologic lesions were seen through 10^{-4} whereas paralysis and weakness was seen only as far as 10^{-2} *Type 2* Again the findings with the Koprowski and Lederle strains correlated with the histopathologic findings With the Sabin strain there is a difference similar to that seen with *Type 1* *Type 3* There is close correlation in the case of the Koprowski strain In the case of the Lederle strain histopathologic findings were seen through the 10^{-5} dilution while weakness and paralysis extended only through 10^{-4} With the Sabin strain histopathologic findings were seen as far as the 10^{-4} dilution while paralysis and weakness was noted only in the 10^{-1} *dilution* group One animal in the 10^{-3} group showed paralysis, but this animal showed no histopathologic lesions of poliomyelitis or any other process and is the only animal in the entire series where weakness and paralysis were noted in the absence of such lesions

for Type 3 No lesions were seen in the case of the Type 2 material; the titer here, as noted, was low in comparison with the other strains tested With the Lederle strains lesions were seen through 10^{4} for Type 1, 10^{5} for Type 2, and 10^{2} for Type 3

c) *General comment* The lesions produced in monkeys by these strains of poliovirus were qualitatively similar to those produced by the virulent strains with which we have experience Chromatolysis of neurons, neuronophagia, and inflammatory cell infiltrate are all seen A certain quantitative trend may be noted in that the morphologic changes induced by the three Sabin strains are less severe than those produced by the other strains. While some of the strains *lack virulence by the intrathalamic route of in*oculation, all were capable of producing lesions when inoculated intraspinally. It is felt that the presence of lesions at sites distant from the site of inoculation may be important in the evaluation of these strains of virus The results reported here, therefore, indicate that there are quantitative differences in the degree of neurovirulence as judged by these methods As will be reported later by Dr. Kirschstein, Figures 1-17 show examples of the lesions seen.*

DEVELOPMENT OF WEAKNESS AND PARALYSIS Effects judged to result from inoculation trauma were seen in nearly all the animals inoculated by the intraspinal route and in an occasional animal inoculated into the thalamus In the former this generally consisted of weakness of one leg and occasionally partial

* See pp. 56 64

TABLE 6 SUMMARY OF COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS TYPE 1

Route	Diln	KOPROWSKI		LEDERLE		SABIN	
		Histologic Findings	Weakness† or Paralysis	Histologic Findings	Weakness† or Paralysis	Histologic Findings	Weakness† or Paralysis
Intraspinal	10^{0}	5/5*	4/5	5/5	5/5	5/5	4/5
	10^{-1}	5/5	5/5	5/5	4/5	5/5	4/5
	10^{-2}	5/5	4/5	5/5	5/5	5/5	2/5
	10^{-3}	4/5	4/5	5/5	4/5	5/5	0/5
	10^{-4}	4/5	4/5	4/5	1/5	2/5	0/5
	10^{-5}	0/5	0/5	0/5	0/5	0/4	0/4
	Threshold PFU/ml	2 6	2 6	1 4	1 4	2 4	4 4
Intrathalamic	10^{0}	2/5	0/5	2/5	0/5	0/0	0/0
	10^{-1}	2/5	0/5	3/5	1/5	0/4	0/4
	10^{-2}	2/5	0/5	3/5	0/5	0/5	0/5
	10^{-3}	2/5	0/5	1/5	0/5	0/3	0/3
	10^{-4}	0/5	0/5	1/5	1/5	0/3	0/3
	10^{-5}	0/5	0/5	—	—	—	—
	Threshold PFU/ml	3 6	>7 6	<2 4	<2 4	>7 4	>7 4

* No with findings/No inoculated
† Only definite progressive weakness or paralysis beyond day 2 scored as positive

TABLE 7 SUMMARY OF COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS TYPE 2

		KOPROWSKI		LEDERLE		SABIN	
ROUTE	DILN	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS
INTRASPINAL	10^{0}	—	—	4/4	3/4	—	—
	10^{-1}	4/4*	3/3	5/5	3/5	—	—
	10^{-2}	0/4	0/4	5/5	5/5	5/5	3/5
	10^{-3}	0/5	0/5	4/5	3/5	4/4	0/4
	10^{-4}	0/4	0/4	3/5	2/5	2/3	0/3
	10^{-5}	—	—	0/5	0/5	0/5	0/5
	Threshold PFU/ml	2 9	2 9	1 5	1 5	2 4	4 4
INTRATHALAMIC	10^{0}	0/5	0/5	4/5	0/5	0/4	0/4
	10^{-1}	0/5	0/5	4/5	0/5	0/5	0/5
	10^{-2}	0/5	0/5	5/5	0/5	0/5	0/5
	10^{-3}	0/5	0/5	4/5	0/5	0/4	0/4
	10^{-4}	0/4	0/4	3/5	1/5	0/4	0/4
	10^{-5}	—	—	1/5	0/5	—	—
	Threshold PFU/ml	4 9	4 9	1 5	1 5	7 4	7 4

* No with findings/No inoculated

† Only definite progressive weakness or paralysis beyond day 2 scored as positive

paralysis; in the case of the latter no more than weakness of one arm and leg was observed These effects were observed one to two days following inoculation Only when there was definite progression of weakness or paralysis after the second day were the animals considered positive Questionable findings were considered negative

Tables 6, 7, and 8 give a comparison between the histopathologic findings and the number of animals showing weakness and paralysis.

a) *Intraspinal inoculation* *Type 1.* Examples of the scoring of inoculated animals are shown in Tables 9, 10, and 11 There was close correlation between progressive weakness and paralysis and the [illegible]

[illegible]

10[illegible] whereas paralysis and weakness was seen only as far as 10^{-3} *Type 2* Again the findings with the Koprowski and Lederle strains correlated with the histopathologic findings. With the Sabin strain there is a difference similar to that seen with Type I *Type 3* There is close correlation in the case of the Koprowski strain In the case of the Lederle strain histopathologic findings were seen through the 10^{-5} dilution while weakness and paralysis extended only through 10^{-3}. With the Sabin strain histopathologic findings were seen as far as the 10^{-4} dilution while paralysis and weakness was noted only in the 10^{-1} dilution group One animal in the 10^{-3} group showed paralysis, but this animal showed no histopathologic lesions of poliomyelitis or any other process and is the only animal in the entire series where weakness and paralysis were noted in the absence of such lesions

TABLE 8 SUMMARY OF COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS TYPE 3

		KOPROWSKI 7 5 PFU/ml		LEDERLE 7 3 PFU/ml		SABIN 7 3 PFU/ml	
ROUTE	DILN	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS	HISTOLOGIC FINDINGS	WEAKNESS† OR PARALYSIS
INTRASPINAL	10^{0}	5/5*	3/5	5/5	4/5	—	—
	10^{-1}	4/4	3/4	5/5	5/5	5/5	3/5
	10^{-2}	5/5	0/5	5/5	4/5	5/5	0/5
	10^{-3}	5/5	2/5	4/4	3/4	3/5	0/5
	10^{-4}	0/5	0/5	2/5	0/5	2/4	0/4
	10^{-5}	1/5	1/5	2/5	0/5	0/4	1/4
	Threshold PFU/ml	<2 5	<2 5	<2 3	3 3	2 3	5 3 ? <2 3 ?
INTRATHALAMIC	10^{0}	3/5	0/5	2/5	1/5	0/10	0/10
	10^{-1}	4/5	0/5	1/5	0/5	0/4	0/4
	10^{-2}	1/5	0/5	1/5	1/5	0/5	0/5
	10^{-3}	2/5	1/5	0/3	0/3		0/5
	10^{-4}	0/5	0/5	0/5	0/5		0/5
	10^{-5}	0/5	0/5	—	—		
	Threshold PFU/ml	3 5	3 5	4 3	4 3	>7 3	>7 3

* No with findings/No inoculated

† Only definite progressive weakness or paralysis beyond day 2 scored as positive

b) *Intrathalamic inoculation* In viewing the tables it is immediately apparent that very few of the animals showing histologic lesions had weakness or paralysis This is in contrast to the findings with respect to the animals inoculated intraspinally

Animals scored as positive were seen after inoculation of the following strains. Lederle 1, 2, and 3, Koprowski 3.

Although the number of positive animals is very small, there does not appear to be a correlation between virus dilution and the presence of weakness or paralysis

VIRUS ISOLATION FROM THE CENTRAL NERVOUS SYSTEM Individual virus isolation studies are being performed on specimens of lumbar cord, cervical cord, and frontal cortex. Thus far the number of animals from which poliovirus has been isolated is considerably lower than the number which showed histologic lesions of poliomyelitis In many instances, virus was isolated at sites distant from the inoculation site

These results are in contrast to our previous experience with virulent polioviruses in which the correlation of virus isolation with histologic lesions has been almost complete

We have prepared this report rather rapidly during the last few days

The summary tables in the report show separate listing for the histologic findings and for the observations for paralysis and weakness (Tables 14 to 19)

I shall purposely gloss over any great elabora-

TABLE 9 PROGRESSIVE WEAKNESS AND PARALYSIS EXAMPLES OF SCORING USED

A certain degree of weakness or paralysis was observed in almost all animals following intraspinal inoculation This was generally a weakness seen in one leg Only when the involvement definitely progressed to complete paralysis after two days or if more than one leg became involved were the animals scored as positive.

In the case of animals inoculated by the intrathalamic route, signs were occasionally observed as early as the second day; these were attributed to the trauma of inoculation Animals showing progressive signs at a later date than this were considered as positive

Representative animals and the scores used are shown below

ANIMAL No	ROUTE	INOCULUM	CLINICAL SIGNS		CONCLUSION
			DAY 2	MAXIMAL	
454	I S	199	W RL	W RL (2)*	Neg
390	I S	199	W RL	PP RL (3)	Neg
498	I S	K-I 10^{-3}	W LL	CP LL (3)	Pos
				CP RL (6)	
311	I S	S-I 10^{5}	RL ?	CP RL (6)	Pos
610	I S	S-III 10^{-3}	W LL	PP LL (10)	Neg (?)
533	I Th	L-II 10^{-4}	Neg	W RL (13)	Pos
637	I Th	S-III 10^{0}	RL *	W RL (3)	Neg
638	I Th	S-III 10^{0}	Neg	Neg	Neg

* First day of test the indicated observation was made

W —Weakness
PP —Partial Paralysis
CP —Complete Paralysis
RL —Right Leg
LL —Left Leg

TABLE 10 INOCULATION TRAUMA INTRASPINALLY INOCULATED ANIMALS

ANIMAL No	INOCULUM	SIGNS		CLASSIFICATION
		DAY 2	MAXIMAL	
262	199	W LL	W LL (2)	Neg
591	199	W RL	W RL (2)	Neg
390	199	W RL	PP RL (3)	Neg
517	199	W RL	W RL (2)	Neg
518	199	Neg	Neg	Neg
453	199	RL ?	W RL (3)	Neg
454	199	W RL	W RL (2)	Neg
655	199	W LL	W LL (2)	Neg
666	199	LL ?	Neg	Neg
719	Virus-10-5	LL ?	W LL (3)	Neg
721	Virus-10-5	PP LL	PP LL (2)	Neg
722	Virus-10-5	W RL	W RL (2)	Neg
723	Virus-10-5	W RL	W RL (2)	Neg

None of the animals showed histological lesions of poliomyelitis
Similar findings noted with 39 other animals inoculated with 10^{-5} dilutions of different strains

TABLE 11 COMPARISON OF WEAKNESS AND PARALYSIS OBSERVED WITH STRAINS OF TYPE I POLIOVIRUS INOCULATED INTRASPINALLY

ANIMAL No	INOCULUM	SIGNS DAY 2	SIGNS MAXIMAL	CLASSIFICATION
507	K-I—10°	W RL	CP RL (9) W LL (3)	Pos
508	K-I—10°	PP LL	CP LL (9) CP RL (5)	Pos
509	K-I—10°	PP LL	CP LL (3) PP RL (7)	Pos
510	K-I—10°	Neg	LL ? (7)	Neg
511	K-I—10°	RL ?	CP RL CP LL (5)	Pos
443	L-I—10°	CP LL CP RL	CP LL CP RL (2)	Pos
444	L-I—10°	CP LL CP RL	CP LL CP RL (2)	Pos
445	L-I—10°	PP LL	CP LL (4) W RL (11)	Pos
446	L-I—10°	PP RL W LL	CP RL. W LL (3)	Pos
447	L-I—10°	W RL	CP RL (3) CP LL (5)	Pos
310	S-I—10°	W RL	CP RL W LL (5)	Pos
311	S-I—10°	RL ?	CP RL (6)	Pos
312	S-I—10°	W RL	CP RL (6)	Pos
313	S-I—10°	W RL	CP RL (4)	Pos
314	S-I—10°	W. LL	PP LL (3)	Neg (?)

tion of the detail presented in these tables I think the methods they carry are self-evident There are differences between these strains.

Individual studies are still being made of tissues that were set aside from animals that were autopsied, and thus far the number of animals from which poliovirus has been isolated is considerably lower than the number which showed histologic lesions of poliomyelitis

In many instances virus was isolated at sites which were distant from the inoculation site, but this work is still going on, and will take some time to complete

We would say, however, that this type of finding in which you find virus with no histologic finding, is in complete contrast to our previous experiences in which the correlation of virus isolation with histologic lesions has been almost complete

We are presenting these findings at this time without any attempt to discuss their significance, but merely to bring these to the attention of the people who are gathered here today.

Dr. Kirschstein will continue with the discussion of the histologic findings which were encountered

Table 12 Examples of Distribution of Lesions

Animal No	Plane	I Sp Inoc Trauma	Histologic Findings						I C.
			Lumbar	Cervical	Thoracic	Lower Medulla	Upper Medulla	Midbrain	Trauma
V-511	1	Present	Sv	Mod Sv	Min	Mild	Mild	Mild	—
	2	Present	Sv	Mild	Neg				
	3	Absent	Sv	Mild	Neg				
	4	Absent	Sv	Min					
	5	Absent	Mod Sv	Min					
	6	Absent	Mild	Min					
V-590	1	—	Mod Sv	Mild	Neg	Mod Sv	Mod Sv	Mod Sv	*Left* Thalamus
	2	—	Mod Sv	Mild	Neg				
	3	—	Mild	Min	Neg				
	4	—	Mild	Neg					*Right* Thalamus
	5	—	Mild	Neg					
	6	—	Min	Neg					

TABLE 11 COMPARISON OF WEAKNESS AND PARALYSIS OBSERVED WITH STRAINS OF TYPE I POLIOVIRUS INOCULATED INTRASPINALLY

ANIMAL NO	INOCULUM	SIGNS DAY 2	SIGNS MAXIMAL	CLASSIFICATION
507	K-I—10°	W RL	CP RL (9) W LL (3)	Pos
508	K-I—10°	PP LL	CP LL (9) CP RL (5)	Pos
509	K-I—10°	PP LL	CP LL (3) PP RL (7)	Pos
510	K-I—10°	Neg	LL ? (7)	Neg
511	K-I—10°	RL ?	CP RL CP LL (5)	Pos
443	L-I—10°	CP LL CP RL	CP LL CP RL (2)	Pos
444	L-I—10°	CP LL CP RL	CP LL CP RL (2)	Pos
445	L-I—10°	PP LL	CP LL (4) W RL (11)	Pos
446	L-I—10°	PP RL W LL	CP RL W LL (3)	Pos
447	L-I—10°	W RL	CP RL (3) CP LL (5)	Pos
310	S-I—10°	W RL	CP RL W LL (5)	Pos
311	S-I—10°	RL ?	CP RL (6)	Pos
312	S-I—10°	W RL	CP RL (6)	Pos
313	S-I—10°	W RL	CP RL (4)	Pos
314	S-I—10°	W LL	PP LL (3)	Neg (?)

tion of the detail presented in these tables I think the methods they carry are self-evident. There are differences between these strains

Individual studies are still being made of tissues that were set aside from animals that were autopsied, and thus far the number of animals from which poliovirus has been isolated is considerably lower than the number which showed histologic lesions of poliomyelitis

In many instances virus was isolated at sites which were distant from the inoculation site, but this work is still going on, and will take some time to complete.

We would say, however, that this type of finding in which you find virus with no histologic finding, is in complete contrast to our previous experiences in which the correlation of virus isolation with histologic lesions has been almost complete.

We are presenting these findings at this time without any attempt to discuss their significance, but merely to bring these to the attention of the people who are gathered here today

Dr. Kirschstein will continue with the discussion of the histologic findings which were encountered

TABLE 15 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 2—INTRASPINAL INOCULATION

DILN	KOPROWSKI 4 9 PFU/ML				LEDERLE 6 5 PFU/ML				SABIN 7 4 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	—	—	—	—	4/4	4/4	4/4	3/4	—	—	—	—
10^{-1}	4/4*	2/4	3/4	3/4	5	5	5	3	—	—	—	—
10^{-2}	0/4	0/4	0/4	0/4	5	5	5	5	5	0	0	3
10^{-3}	0	0	0	0	3	3	3	3	1/4	0/4	0/4	0/4
10^{-4}	0/4	0/4	0/4	0/4	3	3	3	2	2	0	0	0
10^{-5}	—	—	—	—	0	0	0	0	0	0	0	0
Threshold PFU as Log_{10}	2 9	2 9	2 9	2 9	1 5	1 5	1 5	1 5	2 4	>5 4	>5 4	4 4

* No. of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as otherwise indicated

TABLE 16 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 3—INTRASPINAL INOCULATION

DILN	KOPROWSKI 7 5 PFU/ML				LEDERLE 7 3 PFU/ML				SABIN 7 3 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	5*	3	2	3	5	4	4	4	—	—	—	—
10^{-1}	4/4	3	3	3	5	4	5	5	5	2	2	3
10^{-2}	5	1	3	0	5	5	5	4	5	2	1	0
10^{-3}	5	1	3	2	4/4	1	0	3	3	0	0	0
10^{-4}	0	0	0	0	2	1	2	0	2/4	0	0	0
10^{-5}	1	1	1	1	2	2	1	0	0/4	0	0	1
Threshold PFU as Log_{10}	<2 5	<2 5	<2 5	<2 5	<2 3	<2 3	<2 3	3 3	2 3	4 3	4 3	5 3? <2 3?

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as *indicated*

TABLE 13 COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

Type		Intracortical			Intrathalamic		
	Diln	Lumbar Cord	Cervical Cord	Brain Stem	Lumbar Cord	Cervical Cord	Brain Stem
1	10^{0}	2*	2	2	1	2	2
	10^{-1}	0	0	0	2	2	3
	10^{-2}	0	0	0	3	1	3
	10^{-3}	0	0	0	1	2	1
	10^{-4}	0	0	0	1	1	1
3	10^{0}	0	0	0	1	2	2
	10^{-1}	0	0	0	1	1	1
	10^{-2}	0	0	0	1	1	1
	10^{-3}	0	0	0	0/3†	0/3†	0/3†
	10^{-4}	0	0	0	0	0	0

* No of monkeys showing histologic lesions/No of monkeys inoculated
† All groups of 5 monkeys except those noted

TABLE 14 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 1—INTRASPINAL INOCULATION

Diln	Koprowski 7 6 PFU/ml				Lederle 6 4 PFU/ml				Sabin 7 4 PFU/ml			
	L	C	B S	Weakness or Paralysis	L	C	B S	Weakness or Paralysis	L	C	B S	Weakness or Paralysis
10^{0}	5*	5	4	4	5	5	5	5	5	4	3	4
10^{-1}	5	4	5	5	5	3	4	4	5	2	2	4
10^{-2}	5	4	5	4	5	4	4	5	5	3	4	2
10^{-3}	4	3	4	4	5	3	5	4	5	2	3	0
10^{-4}	4	4	4	4	4	3	3	1	2	1	1	0
10^{-5}	0	0	0	0	0	0	0	0	0/4	0/4	0/4	0/4
Threshold PFU as Log_{10}	2 6	2 6	2 6	2 6	1 4	1 4	1 4	1 4	2 4	2 4	2 4	4 4

* No. of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as indicated

TABLE 15 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 2—INTRASPINAL INOCULATION

DILN	KOPROWSKI 4 9 PFU/ML				LEDERLE 6 5 PFU/ML				SABIN 7 4 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^0	—	—	—	—	4/4	4/4	4/4	3/4	—	—	—	—
10^{-1}	4/4*	2/4	3/4	3/4	5	5	5	3	—	—	—	—
10^{-2}	0/4	0/4	0/4	0/4	5	5	5	5	5	0	0	3
10^{-3}	0	0	0	0	3	3	3	3	1/4	0/4	0/4	0/4
10^{-4}	0/4	0/4	0/4	0/4	3	3	3	2	2	0	0	0
10^{-5}	—	—	—	—	0	0	0	0	0	0	0	0
Threshold PFU as Log_{10}	2 9	2 9	2 9	2 9	1 5	1 5	1 5	1 5	2 4	>5 4	>5 4	4 4

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as otherwise indicated

TABLE 16 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 3—INTRASPINAL INOCULATION

DILN	KOPROWSKI 7 5 PFU/ML				LEDERLE 7 3 PFU/ML				SABIN 7 3 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^0	5*	3	2	3	5	4	4	4	—	—	—	—
10^{-1}	4/4	3	3	3	5	4	5	5	5	2	2	3
10^{-2}	5	1	3	0	5	5	5	4	5	2	1	0
10^{-3}	5	1	3	2	4/4	1	0	3	3	0	0	0
10^{-4}	0	0	0	0	2	1	2	0	2/4	0	0	0
10^{-5}	1	1	1	1	2	2	1	0	0/4	0	0	1
Threshold PFU as Log_{10}	<2 5	<2 5	<2 5	<2 5	<2 3	<2 3	<2 3	3 3	2 3	4 3	4 3	5 3? <2 3?

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as indicated

TABLE 13 COMPARATIVE HISTOLOGIC FINDINGS IN RHESUS MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE		INTRACORTICAL			INTRATHALAMIC		
	DILN	LUMBAR CORD	CERVICAL CORD	BRAIN STEM	LUMBAR CORD	CERVICAL CORD	BRAIN STEM
1	10^{0}	2*	2	2	1	2	2
	10^{-1}	0	0	0	2	2	3
	10^{-2}	0	0	0	3	1	3
	10^{-3}	0	0	0	1	2	1
	10^{-4}	0	0	0	1	1	1
3	10^{0}	0	0	0	1	2	2
	10^{-1}	0	0	0	1	1	1
	10^{-2}	0	0	0	1	1	1
	10^{-3}	0	0	0	0/3†	0/3†	0/3†
	10^{-4}	0	0	0	0	0	0

* No of monkeys showing histologic lesions/No of monkeys inoculated
† All groups of 5 monkeys except these noted

TABLE 14 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 1—INTRASPINAL INOCULATION

DILN	KOPROWSKI 7 6 PFU/ML				LEDERLE 6 4 PFU/ML				SABIN 7 4 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	5*	5	4	4	5	5	5	5	5	4	3	4
10^{-1}	5	4	5	5	5	3	4	4	5	2	2	4
10^{-2}	5	4	5	4	5	4	4	5	5	3	4	2
10^{-3}	4	3	4	4	5	3	5	4	5	2	3	0
10^{-4}	4	4	4	4	4	3	3	1	2	1	1	0
10^{-5}	0	0	0	0	0	0	0	0	0/4	0/4	0/4	0/4
Threshold PFU as Log_{10}	2 6	2 6	2 6	2 6	1 4	1 4	1 4	1 4	2 4	2 4	2 4	4 4

* No of monkeys showing lesions of poliomyelitis
Total no. of monkeys 5 except as indicated

TABLE 19 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 3—INTRATHALAMIC INOCULATION

DILN	KOPROWSKI 7 5 PFU/ML				LEDERLE 7 3 PFU/ML				SABIN 7 3 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	1*	2	3	0	1	2	2	1	0/10	0	0	0
10^{-1}	4	4	4	0	1	1	1	0	0/4	0	0	0
10^{-2}	1	1	1	0	1	1	1	1	0	0	0	0
10^{-3}	2	2	2	1	0/3	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	—	—	—	—	—	—	—	—
Threshold PFU as Log_{10}	3 5	3 5	3 5	3 5	4 3	4 3	4 3	4 3	>7 3	>7 3	>7 3	>7 3

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as indicated.

CHAIRMAN ANDERSON Dr Kirschstein

DR KIRSCHSTEIN The lesions produced in monkeys by the strains of poliovirus under discussion are qualitatively essentially the same as those produced by virulent strains Chromatolysis of neurons, neuronophagia and inflammatory cell infiltrate are all seen

The first monkey was inoculated intraspinally We examined six levels on the lumbar enlargement and scored each level for the presence or absence of inoculation trauma Then we scored the lumbar, cervical, thoracic cord, lower medulla, upper medulla, and midbrain individually for the presence or absence of lesions, which are stated to be, either minimal, mild, moderately severe, or, if none were present, negative

In animals inoculated intrathalamically, every animal was checked and again the lesions were scored by the four methods mentioned Certain general statements may be made From this it is obvious that the thoracic cord is quite insensitive to the virus, but rarely, if ever, are lesions produced The lumbar cord in animals inoculated intraspinally, if lesions of polio developed in those animals always showed lesions The cervical cord was quite sensitive

In animals inoculated intrathalamically, the lesions were seen most often in the brainstem, but also in the lumbar area, even more frequently so than in the cervical area

The figures on pages 56 64 will illustrate the type of lesions seen

We would like to summarize by stating that the lesions produced are diagnosable as those of poliomyelitis A certain quantitative trend may be noted in that the morphologic changes induced by the three Sabin strains are less severe than those produced by the other strains

While some of the strains lack virulence by the intrathalamic route of inoculation, all of the strains are capable of producing lesions when inoculated intraspinally It is felt that the presence of lesions at sites distant from the site of inoculation may be important in the evaluation of these strains of virus

CHAIRMAN ANDERSON. The discussion of the paper by Dr Murray and Dr Kirschstein will be held after the presentation of Dr. Melnick's paper The two are so closely related that I think we can save time by having a discussion at that time

TABLE 17 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 1—INTRATHALAMIC INOCULATION

DILN	KOPROWSKI 7 6 PFU/ML				LEDERLE 6 4 PFU/ML				SABIN 7 4 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	2*	2	2	0	1	2	2	0	0/9	0	0	0
10^{-1}	0	0	2	0	2	2	3	1	0/4	0	0	0
10^{-2}	2	2	2	0	3	1	3	0	0	0	0	0
10^{-3}	2	2	2	0	1	2	1	0	0	0	0	0
10^{-4}	0	0	0	0	1	1	1	1	0	0	0	0
10^{-5}	0	0	0	0	—	—	—	—	—	—	—	—
Threshold PFU as Log_{10}	3 6	3 6	3 6	>7 6	<2 4	<2 4	<2 4	<2 4	>7 4	>7 4	>7 4	>7 4

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as indicated

TABLE 18 COMPARATIVE FINDINGS IN MONKEYS INOCULATED WITH ATTENUATED POLIOVIRUS STRAINS

TYPE 2—INTRATHALAMIC INOCULATION

DILN	KOPROWSKI 4 9 PFU/ML				LEDERLE 6 5 PFU/ML				SABIN 7 4 PFU/ML			
	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS	L	C	B S	WEAKNESS or PARALYSIS
10^{0}	0*	0	0	0	4	4	4	0	0	0	0	0
10^{-1}	0	0	0	0	4	4	4	0	0	0	0	0
10^{-2}	0	0	0	0	5	5	5	0	0	0	0	0
10^{-3}	0	0	0	0	4	4	4	0	0	0	0	0
10^{-4}	0/4	0	0	0	3	3	3	1	0	0	0	0
10^{-5}	—	—	—	—	1	1	1	0	—	—	—	-
Threshold PFU as Log_{10}	>4 9	>4 9	>4 9	>4 9	<1 5	<1 5	<1 5	1 5	>7 4	>7 4	>7 4	>7 4

* No of monkeys showing lesions of poliomyelitis
Total no of monkeys 5 except as indicated

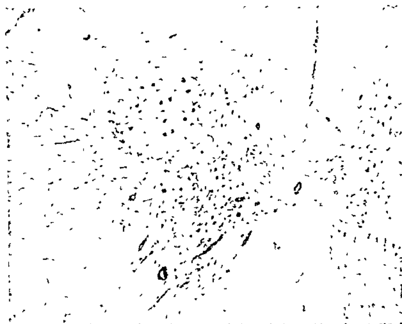


FIG 3 Cervical cord of monkey inoculated intraspinally with Koprowski Type 1 material, undiluted There is little involvement of the anterior horn by inflammatory infiltrate but there is chromatolysis of neurons The infiltrate is in the intermediate area of the gray substance Gallocyanin X27



FIG 4 Lumbar cord of monkey inoculated intraspinally with Lederle Type 1 material, undiluted The area of inoculation trauma and severe involvement of the anterior gray matter is seen with loss of all neurons and dense inflammatory infiltrate Gallocyanin X27

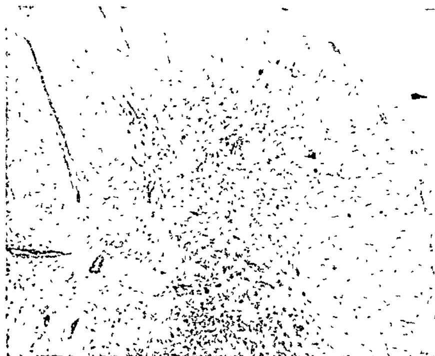


FIG 1 Lumbar cord of monkey inoculated intraspinally with Koprowski Type 1 material, undiluted Note the area of inoculation trauma and an infiltrate in the anterior horn with small foci of neuronophagia Gallocyanin X27

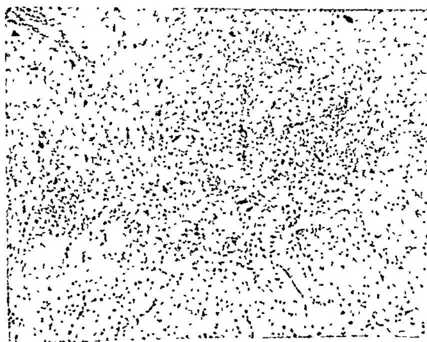


FIG. 2. Lumbar cord of monkey inoculated intraspinally with Koprowski Type 1 material, undiluted. The foci of neuronophagia are seen Gallocyanin X73

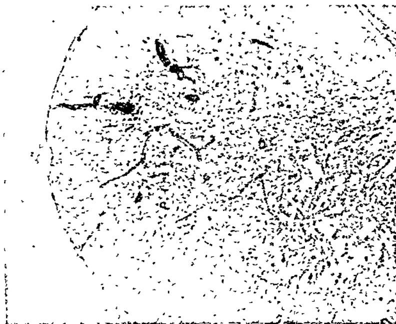


FIG 7 Lumbar cord of monkey inoculated intraspinally with Sabin Type 1 material, undiluted. The area of inoculation trauma and the involvement of the anterior horn are seen There is perivascular cuffing and loss of motor neurons Gallocyanin X27.

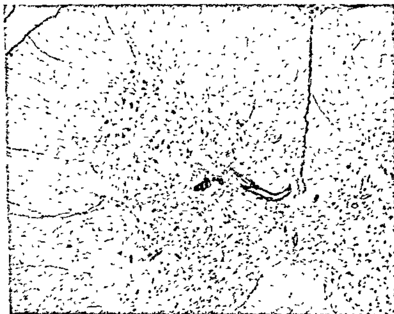


FIG 8 Cervical cord of monkey inoculated intraspinally with Sabin Type 1 material, undiluted Some perivascular cuffing, very mild chromatolysis and little inflammatory infiltrate are seen Gallocyanin X27

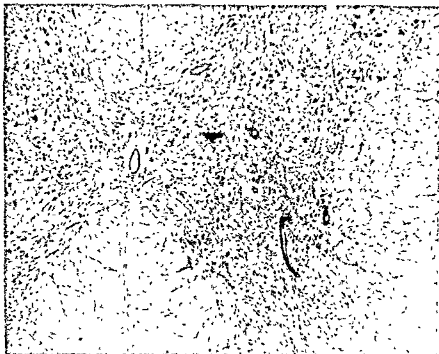


FIG 5 Cervical cord of monkey inoculated intraspinally with Lederle Type 1 material, undiluted There is moderately severe involvement although many neurons are intact Gallocyanin X27



FIG. 6 Upper medulla of monkey inoculated intraspinally with Lederle Type 1 material, undiluted *The cerebellar cortex, the fourth ventricle and choroid plexus are seen in the upper area* The infiltrate is in the vestibular nucleus Gallocyanin X27

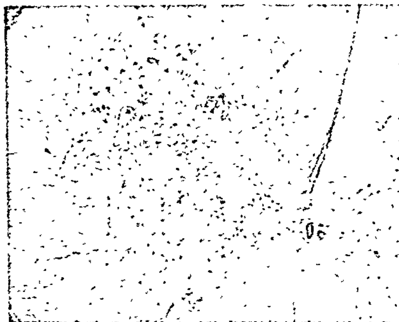


Fig. 11 Lumbar cord of monkey inoculated intrathalamically with Koprowski Type 1 material, undiluted. Several inflammatory infiltrates are seen. Gallocyanin ×27

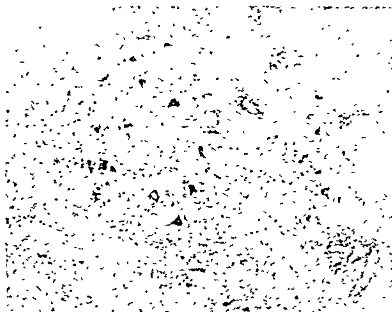


Fig. 12 Lumbar cord of monkey inoculated intrathalamically with Koprowski Type 1 material undiluted. Inflammatory infiltrates and chromatolysis of motor neurons are seen. Gallocyanin ×73

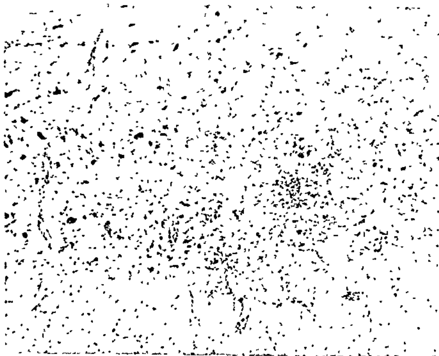


FIG 9 Cervical cord of monkey inoculated intraspinally with Sabin Type I material, undiluted The inflammatory infiltrate and chromatolysis of neurons are well seen Gallocyanin X73

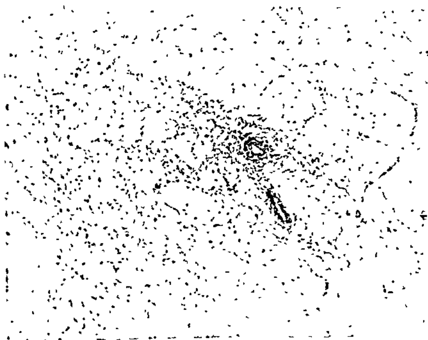


FIG. 10 Red nucleus of monkey inoculated intraspinally with Sabin Type I material, undiluted Marked chromatolysis and an inflammatory infiltrate in the center of the nucleus are seen Gallocyanin X73.

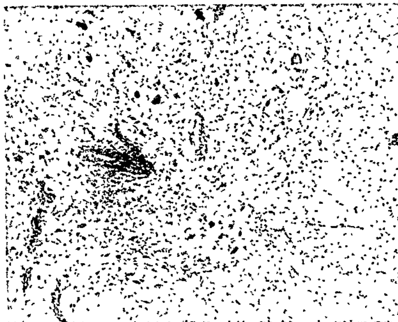


Fig 15 Lumbar cord of monkey inoculated intrathalamically with Lederle Type 1 material, undiluted The perivascular cuffing, chromatolysis and foci of neuronophagia are seen Gallocyanin X73

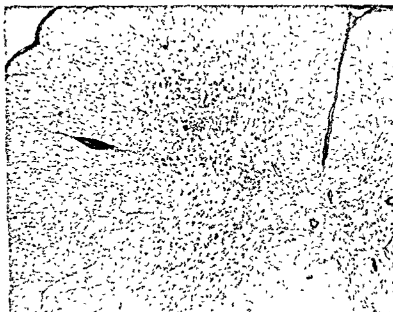


Fig 16 Cervical cord of monkey inoculated intrathalamically with Lederle Type 1 material, undiluted Small infiltrates are seen but many neurons are intact Gallocyanin X27

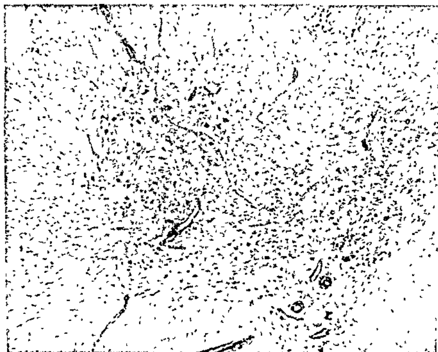


Fig 13 Cervical cord of monkey inoculated intrathalamically *with Koprowski* Type 1 material, undiluted Perivascular cuffing and inflammatory infiltrate in the anterior horn are seen Gallocyanin X27

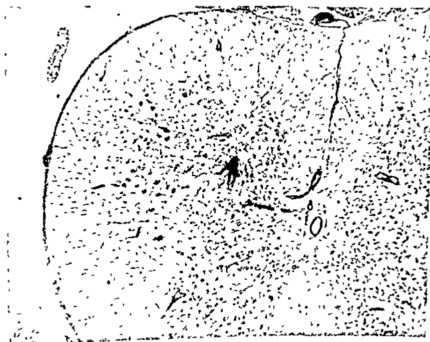


Fig 14 Lumbar cord of monkey inoculated intrathalamically with Lederle Type 1 material, undiluted Perivascular cuffing, infiltrate and chromatolysis are seen Gallocyanin X27

3. MONKEY NEUROVIRULENCE OF ATTENUATED POLIOVIRUS VACCINES BEING USED IN FIELD TRIALS *

JOSEPH L. MELNICK AND JAMES C. BRENNAN

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DR MELNICK (*presenting the paper*) When we started this study about a year ago we were in a bit more difficult position than were Dr Murray and his colleagues when they began their work on the oral poliovirus vaccines[1] The only information available to us at the time was that the Sabin and Lederle-Cox vaccine strains that we were comparing produced disease only in a rare monkey inoculated by the intraspinal route, and almost never, when these strains were inoculated by the intracerebral route As our work progressed, we soon found that we were running into a situation different from that which had been reported by both of these groups previously

Methods of inoculation For intraspinal inoculation, we used a 27-gauge needle, three quarters of an inch in length We selected the fine needle technique because we felt it was important to be sure that 0 1 ml of the material being inoculated would remain within the cord With a 20 gauge needle, especially if retracted after its insertion into the cord, there seems to be a greater opportunity of leakage of the virus inoculum from the site of injection The injection was made into the lumbar enlargement by inserting the needle through the first intervertebral space below the last rib As a measure of a proper inoculation we sought to obtain continuous reflex contractions of the leg muscles during the inoculation In order to achieve this, it was necessary in many monkeys to change the location of the needle during the course of injecting the vaccine

For intracerebral inoculations, we used a 22- or 23 gauge needle, 1¼ inches in length, injecting into the thalamus In some cases we inoculated 0 5 ml into each side of the brain, but later we inoculated the entire 1 0 ml into one thalamic area, because of the amount of trauma that was introduced by the inoculation of both sides of the brain The amount of trauma with the double injection might have been due to the small cynomolgus monkeys used

After a period of observation of about 4 weeks, or less if the monkey was severely paralyzed, the animals were sacrificed and 30 sections of spinal cord were examined, 10 each from the cervical thoracic, and lumbar areas

Scoring system

In order that the data in the tables may be followed, we would like to introduce our scoring system

1 *Severity of lesions* For each hemisection, lesions were graded from 0 to ++++, as follows

- 0 = No lesions in hemisection
- \+ = Single focal neuronal lesion (one or more nerve cells) or single vascular lesion, or single cellular infiltrate of monocytes
- ++ = Combination of two or three of above lesions
- +++ = Clusters of neuronal lesions plus more extensive vascular lesions, and areas of cellular infiltration
- ++++ = Gross destruction of nerve cells, plus extensive areas of infiltration and perivascular cuffing

2 *Score of cord lesions* For one level, the maximum score for both hemisections is thus 8 (++++/++++) For all 10 levels of one area of the cord (lumbar, thoracic, or cervical), the maximum score is 80 In the tables,

* Aided by grants from The National Foundation and from Lederle Laboratories



FIG 17 Cerebellum of monkey inoculated intrathalamically with Lederle Type 1 material The cerebellar cortex and fourth ventricle are seen in the upper area The infiltrate is in one of the cerebellar nuclei Gallocyanin X27

3. MONKEY NEUROVIRULENCE OF ATTENUATED POLIOVIRUS VACCINES BEING USED IN FIELD TRIALS *

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DR MELNICK (*presenting the paper*) When we started this study about a year ago we were in a bit more difficult position than were Dr Murray and his colleagues when they began their work on the oral poliovirus vaccines[1] The only information available to us at the time was that the Sabin and Lederle-Cox vaccine strains that we were comparing produced disease only in a rare monkey inoculated by the intraspinal route, and almost never, when these strains were inoculated by the intracerebral route As our work progressed, we soon found that we were running into a situation different from that which had been reported by both of these groups previously

Methods of inoculation For intraspinal inoculation, we used a 27-gauge needle, three quarters of an inch in length We selected the fine needle technique because we felt it was important to be sure that 0 1 ml of the material being inoculated would remain within the cord With a 20-gauge needle, especially if retracted after its insertion into the cord, there seems to be a greater opportunity of leakage of the virus inoculum from the site of injection The injection was made into the lumbar enlargement by inserting the needle through the first intervertebral space below the last rib As a measure of a proper inoculation we sought to obtain continuous reflex contractions of the leg muscles during the inoculation In order to achieve this, it was necessary in many monkeys to change the location of the needle during the course of injecting the vaccine

For intracerebral inoculations, we used a 22 or 23-gauge needle, 1¼ inches in length, injecting into the thalamus In some cases we inoculated 0 5 ml into each side of the brain, but later we inoculated the entire 1 0 ml. into one thalamic area, because of the amount of trauma that was introduced by the inoculation of both sides of the brain The amount of trauma with the double injection might have been due to the small cynomolgus monkeys used.

After a period of observation of about 4 weeks, or less if the monkey was severely paralyzed, the animals were sacrificed and 30 sections of spinal cord were examined, 10 each from the cervical, thoracic, and lumbar areas

Scoring system

In order that the data in the tables may be followed, we would like to introduce our scoring system

1 *Severity of lesions* For each hemisection, lesions were graded from 0 to ++++, as follows

- 0 = No lesions in hemisection
- \+ = Single focal neuronal lesion (one or more nerve cells) or single vascular lesion, or single cellular infiltrate of monocytes
- ++ = Combination of two or three of above lesions
- +++ = Clusters of neuronal lesions plus more extensive vascular lesions, and areas of cellular infiltration
- ++++ = Gross destruction of nerve cells, plus extensive areas of infiltration and perivascular cuffing

2 *Score of cord lesions* For one level, the maximum score for both hemisections is thus 8 (++++/++++). For all 10 levels of one area of the cord (lumbar, thoracic, or cervical), the maximum score is 80. In the tables,

* Aided by grants from The National Foundation and from Lederle Laboratories

TABLE 1. TYPE 1 LEDERLE VACCINE INOCULATED BY THE INTRACEREBRAL ROUTE

VACCINE INOCULUM (1 ML.)	CYNO NO	WT IN LBS	SIGNS, DAY*	HISTOLOGICAL FINDINGS — POLIO LESIONS† — CERVICAL — CORD	CERVICAL — ROOTS	THORACIC — CORD	THORACIC — ROOTS	LUMBAR — CORD	LUMBAR — ROOTS
Undiluted	93[a]	3	0	9 (0/++)	–	N D		0	–
	94[a]	4	0[b]	3 (0/+)	–	0	–	7 (0/++)	+
	96[a]	4	0	1 (0/+)	–	0	–	2 (0/+)	–
	98[a]	4	0	0	–	0	–	0	–
	99[a]	5	PPA, CPL, Tr, 7	38 (+/++)	–	N D		49 (++/+++)	+
	101[a]	3	PPA, PPRL; Tr, CPLL, 13	33 (+/++)	+	N D		62 (++/++++)	+
	102[a]	5	0[b]	17 (0/+++)	+	9 (0/+++)	–	7 (0/+++)	+
	186	3	0	0	–	0	–	0	–
	187	2	0	2 (0/+)	–	0	–	1 (0/+)	–
	188	4	WL, 5, WLA, 7	19 (0/+++)	–	15 (0/++++)	+	13 (0/+++)	+
	189	4	0	3 (0/++)	+	6 (0/++++)	+	7 (0/+++)	+
10^{-1}	103[a]	5	0	0	–	N D		0	–
	104[a]	6	0	28 (0/+++)	+	19 (0/+++)	+	47 (+/+++)	+
	106[a]	6	0	2 (0/+)	–	2 (0/+)	–	0	–
	108[a]	3	0	3 (0/+)	+	8 (0/++)	+	12 (0/+++)	–
	190	3	Tremors, 13, WA, WL, 14	37 (0/++++)	+	23 (0/++++)	–	37 (0/++++)	+
	191	2	0	0	–	0	–	0	–
	48	4	0	4 (0/+)	+	3 (0/+)	+	18 (0/++)	+
10^{-2}	109[a]	5	Tr, CPA, CPL, 6	47 (++/++++)[c]	–	N D		63 (++/++++)	+
	110[a]	4	0	50 (+/++++)	+	N D		57 (+/++++)	+
	111[a]	6	WRA, PPRL, 6[1]	43 (0/+++)	+	N D		47 (0/++++)	+
	112[a]	4	0[b]	39 (0/+++)	+	21 (0/++)	–	55 (+/++++)	+
	113[a]	3	0[b]	21 (0/+++)	+	N D		51 (+/++++)	+
	114[a]	3	0[b]	47 (0/++++)	+	N D		46 (++/++++)	+
	49	4	PPLL, WRL, 6	4 (0/++)	+	5 (0/++)	+	20 (0/++++)	+
	50	4	PPA, CPL, 7	21 (+/++)	+	N D		51 (++/++++)	+

[1] Monkey 111 also had ptosis of left eye

Note For key references to tables see pp. 100 101

Table 2A Type I Lederle Vaccine Inoculated by the Intraspinal Route. Vaccine Undiluted through 10^{-2}

Vaccine Inoculum (0 1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions† Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots	Needle Tract Lesions in Lumbar Cord**
Undiluted	139	3	CPL, 5	34 (+/+++)	+	N D		47 (0/++++)	+	8
	140	3	CPRL, 5	6 (0/+)	−	N D		51 (0/++++)	+	4, 5
	141	3	CPL, 6	29 (0/++)	−	N D		45 (++/+++)	+	6
	142	4	CPLL, 3, WRL, 6	18 (0/++)	−	N D		51 (0/++++)	+	5
	143	4	CPL, 4	33 (0/+++)	−	N D		57 (++/++++)	+	4–6
	144	3	CPRL, 3, PPLL, 11	11 (0/++)	−	N D		59 (++/++++)	+	2–4
	145	5	CPRL, 4	39 (+/++)	−	N D		47 (+/+++)	+	0
	146	4	0	19 (0/+++)	+	11 (0/+++)	−	43 (0/+++)	+	0
	147	3	0	0	−	0	−	0	+	0
	148	4	CPLL, 6	12 (0/++)	+	N D		31 (0/+++)	+	5
10^{-1}	149	3	CPLL, 3, PPRL, 13, WA, Tremors, 16	28 (0/++)	−	N D		34 (+/+++)	⊕	6
	150	4	CPLL, 3, CPRA, 8	34 (+/++)	+	N D		47 (+/++++)	+	4
	151	3	0	30 (+/+++)	+	29 (0/++)	+	46 (+/+++)	+	4, 5
	152	5	0	32 (+/+++)	−	25 (0/+++)	+	55 (+/+++)	+	2–4
	153	2	WL, 15	29 (0/+++)	+	N D		43 (0/++++)	+	4
	154	3	0	47 (+/+++)	+	42 (+/+++)	+	55 (++/++++)	+	5, 6
10^{-2}	155	2	0	0	−	0	−	0	−	0
	156	4	CPRL, 3	18 (0/+++)	+	N D		58 (+/++++)	+	5
	157	3	CPLL, 3	35 (0/+++)	+	N D		58 (++/++++)	+	5
	158	4	CPRL, 3	15 (0/++)	−	N D		58 (+/++++)	+	4
	159	3	CPLL, 4, PPRL, 13	48 (++/+++)	+	N D		70 (++/++++)	+	4
	160	4	CPLL, 3, WRL, 12	6 (0/+)	−	N D		55 (+/++++)	+	4

Note For key references to tables see pp 100 101

Table 1. Type 1 Lederle Vaccine Inoculated by the Intracerebral Route

Vaccine Inoculum (1 ml.)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings — Polio Lesions† — Cervical		Thoracic		Lumbar	
				Cord	Roots	Cord	Roots	Cord	Roots
Undiluted	93[a]	3	0	9 (0/++)	−	N D		0	−
	94[a]	4	0[b]	3 (0/+)	−	0	−	7 (0/++)	+
	96[a]	4	0	1 (0/+)	−	0	−	2 (0/+)	−
	98[a]	4	0	0	−	0	−	0	−
	99[a]	5	PPA, CPL, Tr, 7	38 (+/++)	−	N D		49 (++/+++)	+
	101[a]	3	PPA, PPRL, Tr, CPLL, 13	33 (+/++)	+	N D		62 (++/++++)	+
	102[a]	5	0[b]	17 (0/+++)	+	9 (0/+++)	−	7 (0/+++)	+
	186	3	0	0	−	0	−	0	−
	187	2	0	2 (0/+)	−	0	−	1 (0/+)	−
	188	4	WL, 5, WLA, 7	19 (0/+++)	−	15 (0/++++)	+	13 (0/+++)	+
	189	4	0	3 (0/++)	+	6 (0/++++)	+	7 (0/+++)	+
10^{-1}	103[a]	5	0	0	−	N D		0	−
	104[a]	6	0	28 (0/+++)	+	19 (0/+++)	+	47 (+/+++)	+
	106[a]	6	0	2 (0/+)	−	2 (0/+)	−	0	−
	108[a]	3	0	3 (0/+)	+	8 (0/++)	+	12 (0/+++)	−
	190	3	Tremors, 13, WA, WL, 14	37 (0/++++)	+	23 (0/++++)	−	37 (0/++++)	+
	191	2	0	0	−	0	−	0	−
	48	4	0	4 (0/+)	+	3 (0/+)	+	18 (0/++)	+
10^{-4}	109[a]	5	Tr, CPA, CPL, 6	47 (++/++++)[c]	−	N D		63 (++/++++)	+
	110[a]	4	0	50 (+/++++)	+	N D		57 (+/++++)	+
	111[a]	6	WRA, PPRL, 6[1]	43 (0/+++)	+	N D		47 (0/++++)	+
	112[a]	4	0[b]	39 (0/+++)	+	21 (0/++)	−	55 (+/++++)	+
	113[a]	3	0[b]	21 (0/+++)	+	N D		51 (+/++++)	+
	114[a]	3	0[b]	47 (0/++++)	+	N D		46 (++/++++)	+
	49	4	PPLL, WRL, 6	4 (0/++)	+	5 (0/++)	+	20 (0/++++)	+
	50	4	PPA, CPL, 7	21 (+/++)	+	N D		51 (++/++++)	+

[1] Monkey 111 also had ptosis of left eye

Note For key references to tables see pp 100 101

Table 2B Type 1 Lederle Vaccine Inoculated by the Intraspinal Route Vaccine 10^{-3} through 10^{-6}

Vaccine Inoculum (0 1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions†: Cervical: Cord	Cervical: Roots	Thoracic: Cord	Thoracic: Roots	Lumbar: Cord	Lumbar: Roots	Needle Tract Lesions in Lumbar Cord**
10^{-3}	386	5	CPL, 3, CPA, 6	53 (++/+++)	–	28 (0/++++)	+	80 (++++/++++)	⊕	1-5T
	387	3	CPL, 3	40 (++/++)	–	45 (+/+++)	+	77 (+++/++++)	+	2, 3
	388	3	CPLL, 3; PPRL, 7	30 (0/++)	+	21 (0/+++)	+	68 (++/++++)	⊕	1-6
	389	3	CPLL, 6, PPRL, 5	20 (0/++)	+	13 (0/++)	–	50 (++/++++)	+	3-8
10^{-4}	381	3	CPLL, PPRL, 5, WA, Tremors, 18	59 (++/+++)	+	58 (0/++++)	+	79 (+++/++++)	⊕	1-9
	382	5	CPLL, 3, CPRL, 6	39 (+/++)	–	23 (0/+++)	+	60 (++/++++)	+	7, 8
	383	3	CPLL, WRL, 5	14 (0/++)	+	9 (0/++)	–	50 (+/++++)	+	2 8
	384	5	CPRL, PPLL, 3	40 (++/++)	+	32 (0/++)	+	67 (++/++++)	⊕	1-7^{T}
	385	4	WL, 5	17 (0/++)	–	22 (0/+++)	+	58 (++/++++)	+	3, 7-9
10^{-5}	396	4	0	0	–	0	–	0	⊕	2-4, 6-10
	397	4	0	0	–	0	–	0	⊕	2, 5^{T}
	398	3	0	0	–	0	–	0	⊕	1-4, 6-8^{T}
	399	3	0	0	–	0	–	0	⊕	2, 9
	400	4	0	0	–	0	–	0	⊕	3, 7
	401	3	0	0	–	0	–	0	⊕	1-3
10^{-6}	402	4	0	0	–	0	–	0	–	0
	403	4	0	0	–	0	–	0	⊕	4
	404	3	0	0	–	0	–	0	⊕	2
	405	4	0	0	–	0	–	0	⊕	3 6
	406	5	0	0	–	0	–	0	⊕	3, 7
	407	4	0	0	–	0	–	0	⊕	1-3

Note For key references to tables see pp. 100 101

this score is the number to the left of the parentheses, the code figure in parentheses indicating the range of severity of the lesions in the cord area under consideration

3 *Spinal root lesions*

- — = *No root lesions present*
- + = Root lesions present anywhere above upper limits of needle injury
- ⊕ = Root lesions present, *but only at or* below spinal cord inoculation site

4 *Needle tract lesions* In the column so headed, the numbers refer to the level or levels where the needle tract lesion was found, for this purpose *the lumbar cord was arbitrarily divided* into 10 consecutive blocks As indicated by a T in some of the tables, needle tract lesions were also found occasionally in the lowest thoracic area of the cord

RESULTS

Table 1 Lederle Type 1 vaccine inoculated by the intracerebral route Clinical signs of poliomyelitis (paralysis, weakness, tremors) were present in each group of monkeys inoculated *through* 10^{3} *The lesions were more* severe in the monkeys injected with the smaller doses of virus, suggesting that the vaccine contains a mixed population *of virus particles differing in* degrees of neurovirulence The CNS lesions extended down into the lumbar cord, severely involving all the animals inoculated with *10* vaccine virus, even the 4 monkeys without clinical disease

Tables 2a, 2b Lederle Type 1 vaccine inoculated by the intraspinal route The same strain, when inoculated by the intraspinal route, produced even more disease and more polio lesions in the spinal cord There were three animals (Nos 147, 155, 402) in whose cords no needle tract lesions could be found, and which did not

eliminated from the final summary of titrations for neurovirulence.

The results of Table 2b show that we do get end points by this method At 10^{-4} concentration of vaccine, all five animals *inoculated came down* with weakness or paralysis In some, not only were the legs involved, but the virus moved up the cord, for in monkey No 381, the arms became involved, and tremors also developed At 10^{-6} and 10^{-4} concentrations, we obtained extensive needle tract damage, but when the virus had been diluted beyond its end point, no weak*ness or paralysis or polio lesions were produced This indicates that the positive results cannot* be due solely to the method of injection without virus multiplication

Table 3 Sabin Type 1 vaccine, inoculated by the intracerebral route Here the animals were almost without response clinically, and only minimal polio lesions were found in two animals that received this material.

Tables 4a, 4b Sabin Type 1 vaccine, inoculated by the intraspinal route When this material was inoculated by the intraspinal route, there was more reaction, particularly in the lumbar area There was involvement in all ten of the inoculated animals with some degree of spread into the cervical area, the spread varying some*what from one animal to the next*

As with the Lederle material, by continuing to dilute Sabin's vaccine we came to an end point On the basis of clinical responses, two of four animals came down at 10^{-2} and none at 10^{-3} concentration A similar fading out of the histological response is evident upon dilution of the vaccine, even though the needle tract lesions were extensive at all dilutions

Table 5 Lederle Type 2 vaccine, inoculated by the intracerebral route This strain was active clinically through the 10^{-2} concentration All *of the 22 monkeys in the titration showed* extensive lesions in the lumbar, thoracic and cervical cords

Tables 6a, 6b Lederle Type 2 vaccine, inoculated by the intraspinal route There was marked neurotropic activity as evidenced by clinical disease and extensive involvement of the spinal cord through 10^{-4} concentration of vaccine Again a spread up into the cervical region was evidenced not only by the lesions in the area but also by weakness and paralysis of the arms, and by tremors, in some of the animals

Again an end point was reached, at 10^{-5} concentration, the injection produced trauma along the needle tract but did not produce either clinical disease or lesions which could be mistaken in any way for those of poliomyelitis

Table 4A Type 1 Sabin Vaccine Inoculated by the Intraspinal Route. Vaccine Undiluted through 10^{-2}

Vaccine Inoculum (0 1 ml.)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings						
				Polio Lesions†						Needle Tract Lesions in Lumbar Cord**
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
Undiluted	161	2	0	3 (0/++)	–	1 (0/+)	–	19 (0/+++)	+	10
	162	2	WRL, 2	2 (0/+)	–	7 (0/++)	–	35 (0/++++)	⊕	2
	163	5	PPLL, 6	5 (0/++)	–	N D		21 (0/++++)	+	4, 5
	164	3	PPL, 6	2 (0/+)	–	N D		38 (0/++++)	+	4-6
	165	3	PPLL, 6	1 (0/+)	–	N D		29 (0/++++)	⊕	3, 4
	166	5	WRA, WRL, 14	21 (0/++)	+	14 (0/++)	+	33 (0/++++)	+	0
	167	4	WLL, 3	22 (0/++)	+	13 (0/+++)	+	56 (++/++++)	+	5-6
	168	3	CPL, 4	8 (0/++)	+	N D		52 (++/++++)	⊕	1 4
	169	2	0	2 (0/+)	–	2 (0/+)	–	23 (0/++++)	⊕	6, 7
	170	2	WL, 4	10 (0/++)	–	10 (0/++)	+	21 (0/++)	+	5
10^{-1}	171	4	CPLL, WLA, 8	17 (0/++)	+	N D		41 (+/++++)	⊕	1 4
	172	2	CPRL, 4	2 (0/+)	–	N D		45 (0/++++)	⊕	3, 4
	173	3	0	0	–	0	–	2 (0/+)	⊕	3
	174	4	CPL, WA, 10, Tr, 11	6 (0/++)	–	N D		46 (0/++++)	⊕	3
	175	2	0	0	–	1 (0/+)	–	0	–	0
	176	3	CPLL, 6	0	–	1 (0/+)	–	13 (0/+++)	+	4
10^{-2}	177	3	CPLL, 3	0	–	0	–	25 (0/+++)	⊕	2 4
	178	2	WLL, 3	14 (0/++)	+	11 (0/+++)	–	36 (0/++++)	⊕	4, 5
	179	5	WLL, 3	0	–	1 (0/+)	–	28 (0/+++)	⊕	1 6
	180	4	WRL, 8	0	–	0	–	20 (0/++++)	+	4-7
	181	5	0	2 (0/++)	–	7 (0/++)	–	33 (0/+++)	⊕	3
	182	3	PPLL, 4	2 (0/+)	–	4 (0/++)	–	41 (0/++++)	+	6-9

Note For key references to tables see pp 100 101

Table 3 Type 1 Sabin Vaccine Inoculated by the Intracerebral Route

Vaccine Inoculum (1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings — Polio Lesions†					
				Cervical		Thoracic		Lumbar	
				Cord	Roots	Cord	Roots	Cord	Roots
Undiluted	116[a]	3	0	0	−	N D		3 (0/++)	+
	117[a]	4	0	0[d]	−	0	−	4 (0/++)	+
	123[a]	6	0	0	−	0	−	0	−
	125[a]	6	0	0	−	0	−	0	−
	183	3	0	0	−	0	−	0	−
	184	4	0	0	−	0	−	0	−
	185	4	0	0	−	0	−	0	−
	51	7	0	0	−	0	−	0	−
	52	6	0	0	−	0	−	0	−
	53	3	0	0	−	0	−	0	−
	54	4	0	0	−	0	−	0	−
	55	3	0	0[d]	−	0[d]	−	0	−
10^{-1}	126[a]	4	0	0	−	0	−	0	−
	129[a]	4	W L, 6	0	−	N D		17 (0/++++)	+
	130[a]	6	0	0	−	N D		0	−
	131[a]	4	0	0	−	N D		0	−
	44	3	0	0	−	0	−	0	−
	45	5	0	0	−	0	−	0	−
	56	3	0	0	−	0	−	0	−
10^{-2}	133[a]	5	0	0	−	N D		0	−
	134[a]	6	0	0	−	N D		0	−
	135[a]	2	0	0	−	0	−	0	−
	126[a]	6	W LL, 16	1 (0/+)	−	7 (0/+++)	+	7 (0/+++)	+
	138[a]	4	0	0	−	0	−	0	−
	46	3	0	0	−	0	−	0	−
	47	4	0	0	−	0	−	0	−

Note For key references to tables see pp 100-101

TABLE 5 TYPE 2 LEDERLE VACCINE INOCULATED BY THE INTRACEREBRAL ROUTE
(1 ml right hemisphere)

Vaccine Inoculum	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings					
				Polio Lesions†					
				Cervical		Thoracic		Lumbar	
				Cord	Roots	Cord	Roots	Cord	Root
Undiluted	219	4	0	15 (0/++)	−	11 (0/++)	−	45 (0/++++)	+
	220	4	Ataxia, 16	20 (0/+++)	+	9 (0/++)	+	28 (0/+++)	+
	221	3	WLL, 17	36 (+/++)	+	29 (0/++)	−	50 (+/++++)	+
	222	3	Clumsy, 14	9 (0/++)	−	12 (0/++++)	+	38 (0/++++)	+
	223	3	0	16 (0/++)	−	6 (0/++)	+	14 (0/++)	−
	224	5	WL, 13	19 (0/++)	−	23 (0/++)	+	42 (0/++++)	+
	225	4	0	10 (0/++)	−	8 (0/++)	+	25 (0/+++)	+
	226	3	CPL, 13	4 (0/++++)	+	21 (0/++++)	+	57 (++/++++)	+
	227	4	0	19 (0/++)	+	0	−	18 (0/++)	+
	228	3	0	19 (0/++)	+	20 (0/+++)	+	17 (0/+++)	+
10^{-1}	229	4	CPRL, PPLL, 13	37 (+/++)	+	33 (0/+++)	+	50 (+/++++)	+
	230	3	WLA, WLL, Tr , 13	39 (0/+++)	+	33 (0/++++)	+	43 (0/+++)	+
	231	3	0	15 (0/++)	−	15 (0/++++)	+	35 (0/++++)	+
	232	3	0	16 (0/++)	−	9 (0/++)	+	25 (0/+++)	+
	233	3	WLA, 15	41 (0/+++)	+	32 (0/++++)	+	43 (0/+++)	+
	234	4	0	13 (0/++)	+	6 (0/++)	+	14 (0/+++)	−
10^{-2}	235	4	WL, 12	44 (+/+++)	+	34 (0/+++)	+	42 (++/+++)	+
	236	3	PPLL, WRL, 12	34 (0/++)	+	28 (0/++)	+	46 (++/++++)	+
	237	4	0	38 (+/++)	+	25 (0/+++)	+	36 (0/+++)	+
	238	4	WL, 13	22 (0/+++)	−	18 (0/++++)	+	16 (0/+++)	+
	239	4	0	21 (0/+++)	−	9 (0/++)	−	20 (0/+++)	+
	240	3	0	0	−	0	−	0	−

Note For key references to tables see pp 100 101

TABLE 4B. TYPE 1 SABIN VACCINE INOCULATED BY THE INTRASPINAL ROUTE VACCINE 10^{3} AND 10^{4}

Vaccine Inoculum (0 1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings						
				Polio Lesions†						Needle Tract Lesions in Lumbar Cord**
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
10^{-3}	377	3	0	0	−	0	−	0	−	9, 10
	378	5	0	0	−	0	−	16 (0/++++)	⊕	1, 3, 7–10
	379	3	WLL, 5	0	−	10 (0/++)	−	34 (0/+++)	+	7, 8
	380	3	PPLL, 3	4 (0/++)	−	3 (0/++)	−	47 (0/++++)	⊕	3-6, 10
10^{-4}	372	3	0	1 (0/+)	−	4 (0/++)	+	40 (0/++++)	+	10
	373	3	0	0	−	0	−	0	−	1
	374	3	0	1 (0/+)	−	0	−	15 (0/+++)	⊕	3-6
	375	2	0	0	−	0	−	0	⊕	6, 7
	376	3	0	16 (0/++)	+	8 (0/++)	−	20 (0/++)	+	2-9

Note For key references to tables see pp 100 101

TABLE 6B TYPE 2 LEDERLE VACCINE INOCULATED BY THE INTRASPINAL ROUTE VACCINE 10^{-3} THROUGH 10^{-5}

Vaccine Inoculum (0.1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions†						Needle Tract Lesions in Lumbar Cord**
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
10^{-3}	419	4	CPRL, 6, CPLL, Tremors, 14, CPA, 15	40 (++/+++)	–	23 (0/+++)	–	64 (++/++++)	⊕	1, 3, 7
	422	5	CPRL, 3, CPLL, 8, WA, 15	33 (0/++)	–	22 (0/++++)	+	65 (++/++++)	+	5
	423	5	CPRL, PPLL, 10	41 (++/+++)	+	39 (0/+++)	–	58 (++/++++)	+	3, 4
10^{-4}	488	4	0	28 (0/++)	+	10 (0/++)	–	35 (0/+++)	+	7-9
	489	3	0	0	–	0	–	1 (0/+)	⊕	7
	490	4	CPLL, 5, CPRL, 6, CPA, 7	40 (++/++)	–	38 (0/++++)	–	56 (++/++++)	⊕	1–4T
	491	4	PPL, 5	31 (0/++)	+	23 (0/++)	+	46 (++/++++)	+	7
10^{-5}	484	4	0	0	–	0	–	0	–	4, 5
	485	4	0	0	–	0	–	0	⊕	1, 2, 6, 7
	386	5	0	0	–	0	–	0	⊕	4-9
	487	3	0	0	–	0	–	0	⊕	1-8

Note For key references to tables see pp 100 101

Table 6A Type 2 Lederle Vaccine Inoculated by the Intraspinal Route Vaccine Undiluted through 10^{-2}

Vaccine Inoculum (0 1 ml)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings — Polio Lesions† — Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots	Needle Tract Lesions in Lumbar Cord**
Undiluted	78	4	CPL, 4	29 (0/+++)	+	27 (0/++++)	+	76 (+/++++)	+	3, 4
	79	5	CPL, 6, WRA, 19	34 (0/+++)	+	36 (0/+++)	+	73 (++/++++)	+	9
	80	3	CPL, 4	18 (0/++)	−	33 (0/++++)	+	74 (+++/++++)	+	5–7
	81	4	CPL, 4, CPA, 8	41 (+/+++)	+	38 (+/+++)	+	78 (+++/++++)	+	3, 4
	82	4	CPL, 11	44 (+/+++)	+	28 (0/+++)	+	74 (+++/++++)	+	8, 9
	83	4	CPRL, 4, PPLL, 11	42 (+/+++)	+	43 (+/+++)	+	53 (+/++++)	+	9
	84	4	CPLL, 4, CPRL, 11	38 (+/+++)	+	35 (0/++++)	+	76 (++/++++)	+	7, 8
	85	4	CPL, 9, PPRA, 12	59 (++/++++)	+	51 (+/++++)	+	62 (0/++++)	⊕	1, 2, 6, 7
	86	3	PPL, WA, Ataxia, 24	32 (0/+++)	+	14 (0/++++)	+	53 (++/++++)	+	6, 7, 10
	87	5	CPL, 3, CPA, Tr, 7	32 (+/++)[c]	−	16 (0/+)	+	74 (++/++++)	+	5, 6
10^{-1}	88	4	CPL, 4	27 (0/++)	+	27 (0/++++)	+	74 (+/++++)	+	4–6
	89	3	CPL, 4	53 (+/+++)	+	57 (0/++++)	+	74 (++/++++)	+	7–9
	90	4	CPL, 4, CPA, 7	44 (++/+++)	+	24 (0/++)	+	72 (++/++++)	+	8, 9
	91	3	CPL, 4, WA, 10	49 (++/+++)	+	57 (++/++++)	+	69 (++/++++)	+	4–6
	209	3	CPL, 4	23 (0/++)	−	19 (0/+++)	+	74 (+++/++++)	+	8–10
	210	3	PPL, 6	31 (0/++)	−	14 (0/++)	+	50 (++/++++)	+	8, 9
10^{-2}	213	4	CPLL, WRL, 17	41 (++/+++)	+	26 (0/++)	+	61 (++/++++)	+	9
	214	3	CPRL, 10	43 (++/+++)	+	24 (0/++++)	+	56 (+/++++)	+	?6–9
	215	4	CPL, 4	32 (0/+++)	−	15 (0/++++)	+	66 (++/++++)	+	4–6
	216	4	CPRL, 4	32 (0/+++)	+	40 (0/++++)	+	62 (++/++++)	+	3–6
	217	3	CPL, WA, 10	18 (0/++)	+	10 (0/+)	−	43 (0/+++)	+	9, 10
	218	3	WRL, 8	34 (0/++)	+	32 (0/+++)	+	58 (+/++++)	+	6, 7

Note For key references to tables see pp 100-101

Table 7 Type 2 Sabin Vaccine Inoculated by the Intracerebral Route
(1 ml right hemisphere)

Vaccine Inoculum	Cy No	Wt in Lbs	Signs, Day*	Histological Findings — Polio Lesions† — Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots
Undiluted	247	3	0	0	–	0	–	0	–
	248	3	0	0	–	0	–	0	–
	249	4	0	0	–	0	–	0	–
	250	4	0	0	–	0	–	0	–
	251	3	0	0	–	0	–	0	–
	252	3	0	0	–	0	–	0	–
	253	4	0	0	–	0	–	0	–
	254	4	0	0	–	0	–	0	–
	255	3	0	0	–	0	–	0	–
	256	4	0	0	–	0	–	0	–
10^{-1}	257	4	0	0	–	0	–	0	–
	258	3	0	0	–	0	–	0	–
	259	5	0	0	–	0	–	0	–
	260	4	0	0	–	0	–	0	–
	261	3	0	0	–	0	–	0	–
	262	5	0	0	–	0	–	0	–
10^{-2}	263	3	0	0	–	0	–	0	–
	264	3	0	0	–	0	–	0	–
	265	4	0	0	–	0	–	0	–
	266	4	0	0	–	0	–	0	–
	267	3	0	0	–	0	–	0	–
	268	4	0	0	–	0	–	0	–

Note For key references to tables see pp 100 101

Table 7. Sabin Type 2 vaccine, inoculated by the intracerebral route This material was clean, producing no clinical reaction and no histological response.

Table 8 Sabin Type 2 vaccine, inoculated by the intraspinal route When inoculated intraspinally, disease was produced through 10^{-1} concentration, and lesions through 10^{-2} It is noteworthy that with this strain there was little spread of virus, as revealed by lesions, from the lumbar to the cervical area Thus, spread seems to be a property of the strain injected and not due to the method of injection

Table 9 Lederle Type 3 vaccine, inoculated by the intracerebral route. Scattered clinical and histopathological activity occurred through the 10^{-1} concentration of vaccine

Tables 10a, 10b Lederle Type 3 vaccine, inoculated by the intraspinal route As with the the other strains, there was much more activity, both clinically and histologically, when the Type 3 vaccine was inoculated by the intraspinal route There was also a considerable spread of virus to the cervical cord

Table 11. Sabin Type 3 vaccine, inoculated by the intracerebral route This strain was free of neurotropic activity by this route

Tables 12a, 12b Sabin Type 3 vaccine, inoculated by the intraspinal route A considerable amount of neurotropic activity was manifested upon intraspinal injection The lesion scores were high, not only in the lumbar area, but also in the cervical area Thus, unlike Sabin's Type 2, his Type 3 vaccine has high capacity for spread up the spinal cord

Table 13 Controls, inoculated by the intraspinal route As control material we inoculated normal tissue culture fluids or ECHO 25 virus by the same method as that used for the vaccines Even though there were in some instances transitory signs due to the damage of the inoculation, the animals then progressed to have a normal appearance within the next week No lesions were found other than those due to the needle tract damage and the spinal root involvement below the site of the needle tract lesion

Tables 14-19 Inoculation trauma and the course of poliomyelitis in individual monkeys These data have been assembled to elucidate any role of inoculation trauma in the subsequent course of the disease due to poliovirus multiplication An arrow indicates a clinical response, one to the left indicates a transitory weakness (W) or paralysis (P), and one to the right indicates progressive, persisting disease through the period of observation

There were many examples of weakness, both transitory and persistent, which occurred as a result of the injection However, development of clinical polio was not particularly associated with prior inoculation trauma, and traumatic weakness did not appear to predispose to a greater susceptibility to poliovirus, contrary to certain views which have been expressed.

Tables 20 and 21. Summary data of inoculation trauma and disease course in intracerebrally and intraspinally inoculated monkeys

With Lederle Type 1 vaccine, 26 animals were inoculated intracerebrally; 11 showed inoculation trauma, which regressed in 3 and persisted in 8 In this group of 26, 8 animals developed clinical poliomyelitis, 6 with persistent paralysis, and 18 of the 26 developed lesions in the lumbar and cervical cord

In 43 monkeys inoculated by the intraspinal route with this vaccine strain, none manifested inoculation trauma, but 25 developed clinical poliomyelitis, and 29 of the 43 showed lesions of poliomyelitis

With Sabin Type 1 material 26 were inoculated intracerebrally, of these 13 showed clinical trauma, but only 2 showed polio lesions and these were relatively mild

However, by the intraspinal route with the Sabin Type 1 vaccine, of the 31 animals inoculated, only two had trauma, yet 19 showed subsequent development of poliomyelitis Of these 11 had paralysis of the legs and in 3 there was additional involvement of the arms, in one, the involvement included the base of the brain, for the animal had ataxia and tremors Of these 31 inoculated monkeys (of which only 2 were traumatized clinically), 26 showed polio lesions in the lumbar cord and in 15 the virus spread and multiplied in the cervical or thoracic cord to produce lesions at these levels

Similarly, the results with Type 2 and 3 vaccines, and with the control materials, indicate that traumatic responses do not confuse, or seem to influence, the response of the monkeys to neurovirulence.

TABLE 9 TYPE 3 LEDERLE VACCINE INOCULATED BY THE INTRACEREBRAL ROUTE
(1 ml right hemisphere)

Vaccine Inoculum	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions† Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots
Undiluted	241	3	WLA, WLL, 4, WRL, 18	30 (0/++)	+	20 (0/++)	−	43 (+/+++)	+
	242	4	0	0	−	0	−	0	−
	243	3	0	0	−	0	−	0	−
	244	4	0	0	−	0	−	0	−
	245	3	WLA, 3, WLL, 15	31 (0/+++)	+	8 (0/++)	+	38 (0/++++)	+
	246	3	0	0	−	0	−	0	−
	296	3	Ataxia, 13	36 (0/++)	−	29 (0/++)	+	47 (++/+++)	+
	297	3	0	0	−	0	−	0	−
	298	4	0	0	−	0	−	0	−
	299	3	0	0	−	0	−	0	−
10^{-1}	300	2	WLA, 7	41 (+/+++)	+	25 (0/++)	+	43 (++/+++)	+
	302	2	0	0	−	0	−	0	−
	303	3	0	0	−	0	−	0	−
	304	3	0	0	−	0	−	0	−
	305	3	0	0	−	0	−	0	−
	306	3	CPL, CPA, tremors, 17	41 (0/+++)	+	47 (+/+++)	+	61 (++/++++)	+
10^{-2}	307	3	0	0	−	0	−	0	−
	308	3	0	0	−	0	−	0	−
	309	3	0	0	−	0	−	0	−
	310	3	0	0	−	0	−	0	−
	311	3	0	0	−	0	−	0	−
	312	3	0	0	−	0	−	0	−

Table 8 Type 2 Sabin Vaccine Inoculated by the Intraspinal Route. Vaccine Undiluted through 10^{-2}

Vaccine Inoculum (0.1 ml.)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings						
				Polio Lesions†						Needle Tract Lesions in Lumbar Cord**
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
Undiluted	77	4	WRL, 7	0	−	0	−	17 (0/++++)	⊕	3, 7, 8
	57	4	PPLL, WRL, 7	1 (0/+)	+	1 (0/+)	−	29 (0/++++)	+	9, 10
	58	3	0	0	−	0	−	13 (0/++++)	+	10
	59	5	WLL, 3	15 (0/++)	+	8 (0/++)	+	27 (0/++++)	+	4-6, 8
	60	3	WLL, 3	1 (0/+)	−	0	−	20 (0/++++)	⊕	2, 4, 5
	61	4	0	0	−	0	−	9 (0/++)	+	0
	62	3	WRL, 3	0	−	0	−	35 (0/++++)	⊕	5-9
	63	3	0	0	−	0	−	0	−	0
	64	4	0	0	−	3 (0/+++)	−	10 (0/+++)	⊕	4, 9[e]
	65	4	0	0	−	0	−	0	−	0[e]
10^{-1}	67	5	0	0	−	0	−	22 (0/+++)	⊕	3
	68	5	WLL, 8	1 (0/+)	−	0	−	32 (0/++++)	⊕	4-6
	69	4	0	0	−	0	−	0	−	0
	70	3	WLL, 3	12 (0/+++)	+	0	−	45 (0/++++)	+	6, 7
	71	3	WLL, 7	0	−	0	−	8 (0/+++)	⊕	8-10
	72	4	CPRL, 4, WRA, 21	17 (0/++)	+	12 (0/+++)	+	37 (0/+++)	⊕	2, 4-10
10^{-2}	73	3	0	0	−	1 (0/+)	−	7 (0/++)	+	3
	74	4	0	0	−	0	−	3 (0/+)	⊕	2, 3, 5
	75	4	0	3 (0/+)	−	2 (0/+)	−	13 (0/+++)	+	0
	76	3	0	0	−	2 (0/+)	−	15 (0/++)	+	9
	211	4	0	0	−	0	−	7 (0/++++)[f]	⊕	7
	212	3	0	0	−	0	−	0	−	0

Note For key references to tables see pp 100-101

TABLE 10B TYPE 3 LEDERLE VACCINE INOCULATED BY THE INTRASPINAL ROUTE. VACCINE 10^{3} AND 10^{4}

Vaccine Inoculum (0 1 ml)	Monkey No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions† — Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots	Needle Tract Lesions in Lumbar Cord**
10^{-3}	Cyn 445	3	CPLL, 4, WRL, 10	0	−	0	−	19 (0/++++)	+	3, 4
	Cyn 446	3	WL, 3	31 (0/++)	+	20 (0/++)	+	36 (0/++++)	+	5
	Rh 427	5	CPLL, 3 WRL, 8	21 (0/++)	−	20 (0/+++)	+	59 (++/++++)	+	4-8
	Rh 428	5	CPRL, 4, CPLL, 21	35 (0/+++)	+	20 (0/++)	+	49 (0/++++)	+	3-5
10^{-4}	Cyn 447	5	0	0	−	0	−	0	−	2
	Cyn 448	4	PPLL, 5	1 (0/+)	−	7 (0/++)	+	39 (0/++++)	+	4-6
	Rh 429	5	0	0	−	0	−	0	⊕	2, 3
	Rh 430	5	0	0	+	0	⊕	8 (0/++)	⊕	3T

Table 10a. Type 3 Lederle Vaccine Inoculated by the Intraspinal Route. Vaccine Undiluted through 10^{-2}

Vaccine Inoculum (0 1 ml.)	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings: Polio Lesions†: Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots	Needle Tract Lesions in Lumbar Cord**
Undiluted	274	3	WLL, 3	18 (0/++)	+	8 (0/++)	+	36 (0/++++)	+	8–10
	275	3	0	2 (0/++)	–	10 (0/+++)	–	35 (0/++++)	+	9, 10
	276	3	PPL, 8, ataxia, 9	58 (++/+++)	+	34 (0/+++)	+	49 (++/+++)	+	7 5
	277	3	WLL, 7	21 (0/++)	+	16 (0/++)	+	43 (+/++++)	+	5, 6
	278	3	WLL, 3	22 (0/+++)	+	8 (0/++)	–	42 (0/++++)	+	5–8
	279	2	0	0	–	0	–	25 (0/++++)	+	7
	280	3	PPL, 9	3 (0/++)	–	0	–	37 (0/++++)	+	2–9
	281	3	PPL, 8	5 (0/++)	–	5 (0/++)	+	40 (0/++++)	+	2–4
	282	3	WRL, 7	8 (0/++)	+	8 (0/++)	–	48 (++/++++)	+	8, 9
	283	4	PPL, 7	26 (0/+++)	+	23 (0/+++)	+	45 (0/++++)	+	9, 10
10^{-1}	284	4	CPL, 4, PPA, 17	37 (0/+++)	+	33 (0/+++)	+	69 (+++/++++)	+	5–8
	285	3	WL, 5	2 (0/++)	–	11 (0/++++)	+	36 (0/++++)	+	9
	286	4	CPLL, WRL, 6	14 (0/++)	–	14 (0/+++)	+	52 (++/++++)	+	7, 8
	287	4	WRL, 6	19 (0/++)	+	3 (0/++)	–	33 (0/+++)	+	9
	288	3	PPLL, WRL, 7	23 (0/+++)	+	6 (0/+++)	+	43 (0/++++)	+	4, 6, 7
	289	3	CPLL, WRL, 7	26 (0/+++)	+	10 (0/+++)	+	48 (0/++++)	⊕	1–8
10^{-2}	290	4	CPL, 5, PPA, *Tr*, *Conv*, 9	44 (++/+++)	+	19 (0/+++)	+	61 (++/++++)	⊕	1, 4–8
	291	3	PPRL, 7	40 (0/+++)	+	18 (0/+++)	+	53 (+/++++)	+	2, 4–7
	292	3	CPL, 4	22 (0/++)	+	17 (0/+++)	+	74 (+++/++++)	+	9, 10
	293	4	CPRL, WLL, 3	43 (++/+++)	+	37 (0/++++)	+	68 (++/++++)	+	9, 10
	294	3	CPL, 4, CPLA, WRA, 10	41 (0/++++)	+	12 (0/++)	+	60 (+/++++)	+	8–9
	295	3	WRL, 3, CPLL, 10	27 (0/+++)	+	27 (0/+++)	+	52 (+/++++)	⊕	1–5

TABLE 10B. TYPE 3 LEDERLE VACCINE INOCULATED BY THE INTRASPINAL ROUTE. VACCINE 10^{-3} AND 10^{-4}

Vaccine Inoculum (0.1 ml.)	Monkey No.	Wt in Lbs.	Signs, Day*	Histological Findings: Polio Lesions† — Cervical: Cord	Cervical: Roots	Thoracic: Cord	Thoracic: Roots	Lumbar: Cord	Lumbar: Roots	Needle Tract Lesions in Lumbar Cord**
10^{-3}	Cyn 445	3	CPLL, 4, WRL, 10	0	–	0	–	19 (0/++++)	+	1, 4
	Cyn 446	3	WL, 4	31 (0/++)	+	20 (0/++)	+	30 (0/++++)	+	5
	Rh 427	5	CPLL, 3, WRL, 8	24 (0/++)	–	20 (0/+++)	+	59 (++/++++)	+	4, 8
	Rh 428	5	CPRL, 4, CPLL, 21	35 (0/+++)	+	20 (0/++)	+	19 (0/++++)	+	3, 5
10^{-4}	Cyn 447	5	0	0	–	0	–	0	–	2
	Cyn 448	4	PPLL, 5	1 (0/+)	–	7 (0/++)	+	39 (0/++++)	+	4, 6
	Rh 429	5	0	0	–	0	–	0	Φ	2, 3
	Rh 430	5	0	0	+	0	Φ	8 (0/++)	Φ	3

TABLE 11. TYPE 3 SABIN VACCINE INOCULATED BY THE INTRACEREBRAL ROUTE
(1 ml right hemisphere)

Vaccine Inoculum	Cyno No	Wt in Lbs	Signs, Day*	Histological Findings — Polio Lesions† — Cervical		Thoracic		Lumbar	
				Cord	Roots	Cord	Roots	Cord	Roots
Undiluted	313	2	0	0	–	0	–	0	–
	314	3	0	0	–	0	–	0	–
	315	2	0	0	–	0	–	0	–
	316	3	0	0	–	0	–	0	0
	318	3	0	0	–	0	–	0	–
	319	3	0	0	–	0	–	0	–
	320	4	0	0	–	0	–	0	–
	321	2	0	0	–	0	–	0	–
	322	3	0	0	–	0	–	0[g]	–
	323	2	0	0	–	0	–	0	–
10^{-1}	324	2	0	0	–	0	–	0	–
	325	3	0	0	–	0	–	0	–
	326	2	0	0	–	0	–	0	–
	327	2	0	0	–	0	–	0[h]	–
	328	3	0	0	–	0	–	0	–
	329	2	0	0	–	0	–	0	–
10^{-2}	330	2	0	0	–	0	–	0	–
	331	2	0	0	–	0	–	0	–
	332	4	0	0	–	0	–	0	–
	333	3	0	0	–	0	–	0	–
	334	3	0	0	–	0	–	0	–
	335	3	0	0	–	0	–	0	–

TABLE 12A TYPE 3 SABIN VACCINE INOCULATED BY THE INTRASPINAL ROUTE VACCINE UNDILUTED THROUGH 10^{-2}

Vaccine Inoculum (0.1 ml.)	Cyno No.	Wt. in Lbs.	Signs, Day*	Histological Findings: Polio Lesions† Cervical Cord	Cervical Roots	Thoracic Cord	Thoracic Roots	Lumbar Cord	Lumbar Roots	Needle Tract Lesions in Lumbar Cord**
Undiluted	270	3	PPLL, WRL, 15, WLA, Conv., 17	32 (0/+++)	+	32 (0/++++)	+	44 (0/++++)	⊕	1 6
	271	2	PPLL, 7, WRL, 15	27 (0/+++)	+	29 (0/+++)	+	40 (0/++++)	+	7, 9
	272	3	PPLL, 2	0	–	1 (0/+)	–	36 (0/+++)	⊕	2 9
	273	3	PPRL, WLL, 6	31 (0/+++)	+	19 (0/+++)	+	43 (0/++++)	⊕	4 10
	336	4	CPL, 6, CPA, tremors, 8	31 (0/+++)	–	21 (0/+++)	+	60 (+/++++)	⊕	1 5
	337	4	WLL, 9, WRL, 16	49 (++/+++)	+	38 (0/+++)	–	46 (0/++++)	+	6 8
	338	3	0	1 (0/+)	–	0	–	19 (0/++++)	⊕	1-4
	339	4	WLL, 8	44 (+/++++)	+	48 (0/++++)	+	50 (++/++++)	+	7, 8
	340	3	CPLL, WLA, 6, CPRL, 23	26 (0/+++)	+	18 (0/++)	+	17 (+/++++)	+	2, 4-6
	341	3	WLL, 8	20 (0/+++)	+	19 (0/++++)	+	38 (0/++++)	⊕	2 8
10^{-1}	342	4	0	0	–	2 (0/++)	+	12 (0/++++)	⊕	10
	343	4	0	6 (0/++)	–	6 (0/++)	–	14 (0/++)	+	0
	344	3	PPLL, 6	0	–	3 (0/++)	–	39 (0/+++)	⊕	1-9
	345	3	0	0	–	3 (0/++)	–	23 (0/+++)	–	1, 4
	346	3	CPLL, WRL, 8	8 (0/++)	–	11 (0/++)	+	45 (0/++++)	⊕	1-9
	347	4	CPRL, 8	7 (0/++)	–	18 (0/+++)	+	54 (++/++++)	+	3 5
10^{-2}	348	4	PPL, 8	18 (0/++)	+	18 (0/+++)	+	50 (++/++++)	+	7 2
	349	4	0	0	–	0	–	5 (0/+++)	⊕	9
	350	3	0	15 (0/+++)	+	2 (0/++)	–	21 (0/++++)	⊕	6, 7
	351	3	CPRL, 9, PPLL, 11	41 (++/++++)	+	34 (0/+++)	+	54 (0/++++)	+	2-6, 8
	352	3	0	0	–	0	–	17 (0/+++)	⊕	7-9
	353	3	CPLL, 9	0	–	8 (0/++++)	+	51 (+/++++)	+	7 6, 7

TABLE 12B. TYPE 3 SABIN VACCINE INOCULATED BY THE INTRASPINAL ROUTE VACCINE 10^{-3} AND 10^{-4}

VACCINE INOCULUM (0 1 ML)	MONKEY NO	WT IN LBS	SIGNS, DAY*	HISTOLOGICAL FINDINGS						NEEDLE TRACT LESIONS IN LUMBAR CORD**
				POLIO LESIONS†						
				CERVICAL		THORACIC		LUMBAR		
				CORD	ROOTS	CORD	ROOTS	CORD	ROOTS	
10^{-3}	Cyn 441	3	0	0	–	1 (0/+)	–	13 (0/++++)	⊕	7
	Cyn 442	5	0	0	–	0	–	0	⊕	7
	Rh 391	5	0	0	–	0	–	3 (0/++)	+	5
	Rh 394	5	0	2 (0/+)	–	0	–	25 (0/+++)	⊕	2–4
10^{-4}	Cyn 443	4	0	0	–	0	–	0	⊕	9
	Cyn 444	4	0	0	–	0	–	0	⊕	1–2, 4–10
	Rh 395	5	0	0	–	0	–	0	⊕	1, 7–9
	Rh 426	5	0	0	–	0	–	0	⊕	5

TABLE 13 CONTROL TISSUE CULTURE FLUIDS AND ECHO 25 VIRUS INOCULATED BY THE INTRASPINAL ROUTE

Inoculum[k] (0.15 ml.)	Cyno No.	Wt in Lbs	Signs, Day*	Histological Findings						
				Polio Lesions†						Needle Tract Lesions in Lumbar Cord**
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
Tissue culture fluids										
Lactalbumin medium (M-E) without serum, taken from normal monkey kidney cultures after 5 days' contact at 37° as maintenance medium	354	3	0	0[q]	–	0[q]	–	0	⊕	6, 7
	355	3	0	0	–	0	–	0	⊕	2, 5, 9
	356	4	0	0	–	0	–	0	⊕	6
	357	4	0[m]	0	–	0	–	0	⊕	3, 7, 8, 10
	358	4	0	0	–	0	–	0	⊕	1-6
	359	4	0[p]	0	–	0	–	0	⊕	2, 4-10[T]
ECHO 25										
Undiluted	409	4	0	0	–	0	–	0	⊕	1, 4[T]
	420	5	0	0	–	0	–	0	⊕	1

TABLE 12B TYPE 3 SABIN VACCINE INOCULATED BY THE INTRASPINAL ROUTE VACCINE 10^{-3} AND 10^{-4}

Vaccine Inoculum (0.1 ml.)	Monkey No.	Wt. in Lbs.	Signs, Day*	Histological Findings						Needle Tract Lesions in Lumbar Cord**
				Polio Lesions†						
				Cervical		Thoracic		Lumbar		
				Cord	Roots	Cord	Roots	Cord	Roots	
10^{-3}	Cyn 441	3	0	0	–	1 (0/+)	–	13 (0/++++)	⊕	7
	Cyn 442	5	0	0	–	0	–	0	⊕	7
	Rh 391	5	0	0	–	0	–	3 (0/++)	+	5
	Rh 394	5	0	2 (0/+)	–	0	–	25 (0/+++)	⊕	2–4
10^{-4}	Cyn 443	4	0	0	–	0	–	0	⊕	9
	Cyn 444	4	0	0	–	0	–	0	⊕	1–2, 4–10
	Rh 395	5	0	0	–	0	–	0	⊕	1, 7–9
	Rh 426	5	0	0	–	0	–	0	⊕	5

TABLE 15. INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

CONC OF TC FLUID AND CYNO NO	INOC TRAUMA	POLIO COURSE OF DISEASE
Undil		
116*	W ←	O
117*	W ←	O
123*	O	O
125*	W ←	O
183	P ←	O
184	O	O
185	O	O
51	O	O
52	O	O
53	W ←	O
54	O	O
55	W ←	O
10^{-1}		
126*	O	O
129*	W →	W →
130*	O	O
131*	O	O
44	O	O
45	O	O
56	W ←	O
10^{-2}		
133*	W ←	O
134*	W →	O
135*	O	O
136*	W ←	W →
138*	W →	O
46	O	O
47	W ←	O

Data from Table 3
Sabin Type 1, IC

CONC OF TC FLUID AND CYNO NO	INOC TRAUMA	POLIO COURSE OF DISEASE
Undil		
161	O	O
162	O	W →
163	O	P →
164	O	P →
165	O	P →
166	O	W →
167	O	W →
168	O	P →
169	O	O
170	O	W ←
10^{-2}		
171	O	P →
172	O	P →
173	W ←	O
174	O	P →
175	O	O
176	O	P →
10^{-4}		
177	O	P →
178	O	W →
179	O	W →
180	O	W →
181	O	O
182	O	P →

Data from Table 4a
Sabin Type 1, IS

CONC OF TC FLUID AND CYNO NO	INOC TRAUMA	POLIO COURSE OF DISEASE
10^{-3}		
377	O	O
378	O	O
379	O	W ←
380	O	P →
10^{-4}		
372	O	O
373	O	O
374	O	O
375	O	O
376	W ←	O

Data from Table 4b
Sabin Type 1, IS

TABLE 14 INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
93*	W ←	O
94*	W →	O
96*	W →	O
98*	W ←	O
99*	O	P →
101*	W →	P →
102*	W →	O
186	O	O
187	O	O
188	O	W ←
189	O	O
10^{-1}		
103*	O	O
104*	O	O
106*	W →	O
108*	O	O
190	O	W →
191	O	O
48	O	O
10^{-2}		
109*	O	P →
110*	O	O
111*	W →	P →
112*	W →	O
113*	W ←	O
114*	W →	O
49	O	P →
50	O	P →

Data from Table 1
Lederle Type 1, IC

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
139	O	P →
140	O	P →
141	O	P →
142	O	P →
143	O	P →
144	O	P →
145	O	P →
146	O	O
147	O	O
148	O	P →
10^{-1}		
149	O	P →
150	O	P →
151	O	O
152	O	O
153	O	W →
154	O	O
10^{-2}		
155	O	O
156	O	P →
157	O	P →
158	O	P →
159	O	P →
160	O	P →

Data from Table 2a
Lederle Type 1, IS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
10^{-3}		
386	O	P →
387	O	P →
388	O	P →
389	O	P →
10^{-4}		
381	O	P →
382	O	P →
383	O	P →
384	O	P →
385	O	W →
10^{-5}		
396	O	O
397	O	O
398	O	O
399	O	O
400	O	O
401	O	O
10^{-6}		
402	O	O
403	O	O
404	O	O
405	O	O
406	O	O
407	O	O

Data from Table 2b
Lederle Type 1, IS

TABLE 17 INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio. Course of Disease
Undil		
247	W →	O
248	W →	O
249	W →	O
250	W ←	O
251	O	O
252	O	O
253	O	O
254	W →	O
255	W ←	O
256	O	O
10^{-1}		
257	W ←	O
258	W →	O
259	W →	O
260	W →	O
261	O	O
262	W →	O
10^{-2}		
263	O	O
264	O	O
265	O	O
266	O	O
267	O	O
268	O	O

Data from Table 7
Sabin Type 2, IC

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
77	O	W →
57	O	P →
58	O	O
59	O	W →
60	O	W →
61	O	O
62	O	W →
63	O	O
64	O	O
65	O	O
10^{-1}		
67	O	O
68	O	W →
69	O	O
70	O	W →
71	O	W ←
72	O	P →
10^{-2}		
73	O	O
74	O	O
75	O	O
76	O	O
211	O	O
212	O	O

Data from Table 8
Sabin Type 2, IS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio-Course of Disease
Normal TC fluid		
354	O	O
355	O	O
356	O	O
357	W ←	O^{m}
358	O	O
359	P ←	O^{p}
ECHO-25 Undil		
409	O	O
420	O	O

Data from Table 13
Controls, IS

TABLE 16. INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
219	W ←	O
220	O	P ←
221	W →	W →
222	O	W ←
223	O	O
224	W →	W →
225	O	O
226	W →	P →
227	O	O
228	W →	O
10^{-1}		
229	O	P →
230	W →	W →
231	W ←	O
232	O	O
233	O	W →
234	O	O
10^{-2}		
235	O	W →
236	W →	P →
237	O	O
238	W →	W ←
239	O	O
240	O	O

Data from Table 5
Lederle Type 2, IC

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
78	O	P →
79	O	P →
80	O	P →
81	O	P →
82	O	P →
83	O	P →
84	O	P →
85	O	P →
86	O	P →
87	O	P →
10^{-1}		
88	O	P →
89	O	P →
90	O	P →
91	O	P →
209	O	P →
210	O	P →
10^{-2}		
213	O	P →
214	O	P →
215	O	P →
216	O	P →
217	O	P →
218	O	W →

Data from Table 6a
Lederle Type 2 IS

Conc of TC Fluid and Cyno No	Inoc. Trauma	Polio Course of Disease
10^{-3}		
419	O	P →
422	O	P →
423	O	P →
10^{-4}		
488	O	O
489	O	O
490	W ←	P →
491	O	P →
10^{-5}		
484	O	O
485	W ←	O
486	W ←	O
487	O	O

Data from Table 6b
Lederle Type 2, IS

TABLE 19 INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
313	0	0
314	W ←	0
315	0	0
316	0	0
318	0	0
319	W →	0
320	0	0
321	0	0
322	0	0
323	W ←	0
10^{-1}		
324	W ←	0
325	0	0
326	0	0
327	W ←	0
328	W →	0
329	0	0
10^{-2}		
330	0	0
331	0	0
332	W ←	0
333	W ←	0
334	0	0
335	0	0

Data from Table 11
Sabin Type 3, IC

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
270	0	P →
271	0	P →
272	0	P →
273	0	P →
336	0	P →
337	0	W →
338	0	0
339	0	W →
340	0	P →
341	0	W →
10^{-1}		
342	W ←	0
343	0	0
344	0	P →
345	0	0
346	0	P →
347	0	P →
10^{-2}		
348	0	P →
349	0	0
350	0	0
351	0	P →
352	0	0
353	0	P →

Data from Table 12a
Sabin Type 3, IS

Conc of TC Fluid and Monkey[1] No	Inoc Trauma	Polio: Course of Disease
10^{-3}		
441	0	0
442	0	0
391(Rh)	0	0
394(Rh)	0	0
10^{-4}		
443	0	0
444	0	0
395(Rh)	W ←	0
426(Rh)	0	0

Data from Table 12b
Sabin Type 3, IS

TABLE 18 INOCULATION TRAUMA & THE COURSE OF POLIOMYELITIS IN INDIVIDUAL MONKEYS

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio. Course of Disease
Undil		
241	O	W →
242	O	O
243	O	O
244	O	O
245	O	W →
246	O	O
296	O	P ←
297	O	O
298	W →	O
299	O	O
10^{-1}		
300	O	W →
302	O	O
303	O	O
304	O	O
305	O	O
306	O	P →
10^{-2}		
307	O	O
308	W →	O
309	O	O
310	O	O
311	O	O
312	O	O

Data from Table 9
Lederle Type 3, IC

Conc of TC Fluid and Cyno No	Inoc Trauma	Polio Course of Disease
Undil		
274	O	W →
275	O	O
276	O	P →
277	O	W ←
278	O	W →
279	O	O
280	O	P →
281	O	P →
282	O	W →
283	O	P →
10^{-1}		
284	O	P →
285	O	W ←
286	O	P →
287	O	W →
288	O	P →
289	W →	P →
10^{-2}		
290	O	P →
291	O	P →
292	O	P →
293	O	P →
294	O	P →
295	W ←	P →

Data from Table 10a
Lederle Type 3, IS

Conc of TC Fluid and Monkey[1] No	Inoc. Trauma	Polio Course of Disease
10^{-3}		
445	P →	P →
446	O	W →
427(Rh)	O	P →
428(Rh)	O	P →
10^{-4}		
447	O	O
448	O	P →
429(Rh)	O	O
430(Rh)	W ←	O

Data from Table 10b
Lederle Type 3, IS

TYPE 2								
Lederle, IC (Table 5)	22	9 7W → 2W ←	11	5W → 2W ←	3P → 1P ←		21	21
Lederle, IS (Table 6a, b)	33	3 3W ←	27	1W →	26P →	13A (3M)	28	28
Sabin, IC (Table 7)	22	11 8W → 3W ←	0				0	0
Sabin, IS (Table 8)	22	0	9	6W → 1W ←	2P →	1A	1	17

TABLE 20 SUMMARY DATA OF INOCULATION TRAUMA AND DISEASE COURSE IN INTRACEREBRALLY AND INTRASPINALLY INOCULATED MONKEYS TYPE 1 AND TYPE 2

(For intraspinally inoculated groups, the spread of virus in the spinal cord is indicated)

Vaccine Strain and Route of Inoc	Number of Cynomolgus Monkeys[t]							
	Inoculated	With Inoculation Trauma[r]	Undergoing Attacks of Poliomyelitis				With Cord Lesions of Poliomyelitis[u]	
			Tot	Weakness	Paralysis	Spread of Virus From Lumbar Area in IS-Inoc Monkeys	Cervical and/or Thoracic	Lumbar
TYPE 1								
Lederle, IC (Table 1)	26	11 8W → 3W ←	8	1W → 1W ←	6P →		18	18
Lederle, IS (Table 2a, b)	43	0	25	1W → 1W ←	23P →	4A (2M)[v]	29	29
Sabin, IC (Table 3)	26	13 3W → 9W ← 1P ←	2	2W →			1	3
Sabin, IS (Table 4a, b)	31	2 2W ←	19	6W → 2W ←	11P →	3A (1M)	15	26

TABLE 21 SUMMARY DATA OF INOCULATION TRAUMA AND DISEASE COURSE IN INTRACEREBRALLY AND INTRASPINALLY INOCULATED MONKEYS. TYPE 3 AND CONTROLS

(For intraspinally inoculated groups, the spread of virus in the spinal cord is indicated.)

Vaccine Strain and Route of Inoc	Number of Monkeys[t]							
	Inoculated	With Inoculation Trauma[t]	Undergoing Attacks of Poliomyelitis				With Cord Lesions of Poliomyelitis[u]	
			Tot	Weakness	Paralysis	Spread of Virus From Lumbar Area in IS-Inoc Monkeys	Cervical and/or Thoracic	Lumbar
Type 3								
Lederle, IC (Table 9)	22	2 2W →	5	3W →	1P → 1P ←		5	5
Lederle, IS (Table 10a, b)	30	4 1W → 2W ← 1P →	25	5W → 2W ←	18P →	4A (2M)[v]	24	27
Sabin, IC (Table 11)	22	8 2W → 6W ←	0				0	0
Sabin, IS (Table 12a, b)	30	2 2W ←	15	3W →	12P →	3A (2M)	15	24

TABLE 23 COMPARATIVE TITRATIONS FOR NEUROVIRULENCE OF TYPE 2 CANDIDATES FOR AN ORAL POLIOVIRUS VACCINE

Route of Inoc	Conc of TC Fluid	Monkeys Which Received Lederle-Cox Strain			Monkeys Which Received Sabin Strain		
		Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[1]	Developing Cord Lesions of Polio	Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[1]	Developing Cord Lesions of Polio
Intracerebral	Undil	6.9	5/10[2]	10/10	7.2	0/10	0/10
	10^{-1}	5.9	1/6	6/6	6.2	0/6	0/6
	10^{-2}	4.9	3/6	5/6	5.2	0/6	0/6
Intraspinal	Undil	5.9	10/10	10/10	6.2	5/8	8/8
	10^{-1}	4.9	6/8	6/6	5.2	4/5	5/5
	10^{-2}	3.9	6/6	6/6	4.2	0/5	4/5
	10^{-3}	2.9	3/3	3/3			
	10^{-4}	1.9	2/4	3/4			
	10^{-5}	0.9	0/4	0/4			

[1] Progressive weakness or paralysis beyond the second day after inoculation
[2] Numerator = number of monkeys positive
Denominator = number of monkeys inoculated

TABLE 24 COMPARATIVE TITRATIONS FOR NEUROVIRULENCE OF TYPE 3 CANDIDATES FOR AN ORAL POLIOVIRUS VACCINE

Route of Inoc	Conc of TC Fluid	Monkeys Which Received Lederle-Cox Strain			Monkeys Which Received Sabin Strain		
		Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[1]	Developing Cord Lesions of Polio	Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[1]	Developing Cord Lesions of Polio
Intracerebral	Undil	7.2	3/10[2]	3/10	7.5	0/10	0/10
	10^{-1}	6.2	2/6	2/6	6.5	0/6	0/6
	10^{-2}	5.2	0/6	0/6	5.5	0/6	0/6
Intraspinal	Undil	6.2	8/10	10/10	6.5	9/10	10/10
	10^{-1}	5.2	6/6	6/6	5.5	3/6	6/6
	10^{-2}	4.2	6/6	6/6	4.5	3/6	6/6
	10^{-3}	3.2	4/4	3/4	3.5	0/4	2/4
	10^{-4}	2.2	1/4	2/4	2.5	0/4	0/4

[1] Progressive weakness or paralysis beyond the second day after inoculation
[2] Numerator = number of monkeys positive
Denominator = number of monkeys inoculated

TABLE 22 COMPARATIVE TITRATIONS FOR NEUROVIRULENCE OF TYPE 1 CANDIDATES FOR AN ORAL POLIOVIRUS VACCINE[1]

Route of Inoc	Conc of TC Fluid	Monkeys Which Received Lederle-Cox Strain			Monkeys Which Received Sabin Strain		
		Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[2]	Developing Cord Lesions of Polio	Log TCD_{50} Inoc	Developing Weakness or Paralysis of Polio[2]	Developing Cord Lesions of Polio
Intracerebral	Undil	7 3	3/11[3]	6/11	7 3	0/12	1/12
	10^{-1}	6 3	1/7	4/7	6 3	1/7	1/7
	10^{-2}	5 3	4/8	8/8	5 3	1/7	1/7
Intraspinal	Undil	6 3	8/9	9/9	6 3	8/10	10/10
	10^{-1}	5 3	3/6	6/6	5 3	4/5	4/5
	10^{-2}	4 3	5/5	5/5	4 3	5/6	6/6
	10^{-3}	3 3	4/4	4/4	3 3	2/4	3/4
	10^{-4}	2 3	5/5	5/5	2 3	0/5	3/5
	10^{-5}	1 3	0/6	0/6			
	10^{-6}	0 3	0/5	0/5			

[1] Of 46 control monkeys inoculated intracerebrally, and 28 inoculated intraspinally, none developed disease or lesions
[2] Progressive weakness or paralysis beyond the second day after inoculation
[3] Numerator = number of monkeys positive
Denominator = number of monkeys inoculated

Tables 22-25 Comparative titrations for neurovirulence of candidates for an oral poliovirus vaccine

The data which have been detailed earlier for responses in individual monkeys are summarized in the following tables In addition to calculating the titers in conventional fashion we have added, in Table 25, the sum of the polio lesion scores of the individual monkeys used in the titrations This adds another parameter to the comparison, for it indicates the severity of the lesions and in addition it measures the capacity of the strain to spread in the spinal cord away from its site of inoculation The term MPD_{50} is used here (Table 25) indicating monkey poliomyelitis doses, i e., the dose which produced specific lesions of poliomyelitis in 50% of the inoculated monkeys Usually, as shown in Table 22, the titer based on lesions was about 0.5 to 1 0 $\log_{10}$ units higher than that based on the appearance of paralysis and weakness

For Type 1, the Lederle material contained more than 300 MPD_{50} per ml by the intracerebral route and 300 000 MPD_{50} per ml by the intraspinal route (see Tables 22 and 25) Sabin's strain produced a significant response in only 2 monkeys by the intracerebral route but the intraspinal titer proved to be 100 000 MPD_{50} per ml The extent of multiplication is also evidenced by the summed lesion scores presented in Table 25 After intracerebral injection, Lederle's strain produced marked destruction in both cervical and lumbar areas, as shown by the high scores of 457 and 619, while Sabin's strain yielded a score of only 24. After intraspinal inoculation in the lumbar area, the Lederle material showed a high degree of activity (lumbar score, 1597) and spread into the cervical area (score of 844)—ratio of cervical to lumbar scores, about ½ Sabin's material showed less but significant spread (ratio of cervical to lumbar scores, 123/837, or about 1/7). A low ratio, as 1/7, of cervical to lumbar scores indicates little spread of the virus from the area of in-

jection while a high ratio, as ½, indicates extensive spread of neurovirulent virus to the cervical area

For Type 2, Lederle's strain titered over 300 MPD_{50} per ml by the intracerebral route and 200,000 MPD_{50} by the intraspinal route, while Sabin's strain was negative intracerebrally but had a titer of at least 2500 MPD_{50} by the intraspinal route (see Tables 23 and 25). After intraspinal inoculation, Lederle's strain showed a high degree of activity (lumbar lesion score, 1778) and of spread (cervical score 1009) with a ratio of cervical to lumbar scores approximately ½, in contrast to the relatively low degree of activity and spread of Sabin's strain (ratio of cervical to lumbar scores, 44/346, or about ⅛)

For Type 3, Lederle's strain contained only 1 MPD_{50} per ml by the intracerebral route, but had a titer of 10 000 MPD_{50} by the intraspinal route (See Tables 24 and 25). After intraspinal inoculation, the differences in lesion scores between Type 3 strains were less marked than for the other strains, and the degree of spread was also similar, a high ratio of cervical to lumbar scores, about ½, being found for both strains (Lederle, 584/1259, Sabin 355 850)

A further summation of our results is given in Table 26 We have considered 0 01 ml of infectious tissue culture fluid containing 10^7 TCD_{50}, as the dose generally used for human feeding with Sabin's strains, and 10^5 to 10^6 TCD_{50} as the dose with Lederle's strains Thus one human dose of virus, the amount used to produce infection in the vaccinated child

TABLE 26. AMOUNT OF TCD_{50}, $MPD_{50}(IC)$[1] AND $MPD_{50}(IS)$[2] IN ONE HUMAN DOSE OF VACCINE

VACCINE STRAIN		TCD_{50}	$MPD_{50}(IC)$	$MPD_{50}(IS)$
Lederle	1	10^5–10^6	$10^{0.5}$–$10^{1.7}$	$10^{3.5}$–$10^{4.6}$
	2	10^5–10^6	$10^{0.6}$–$10^{1.6}$	$10^{3.3}$–$10^{4.3}$
	3	10^5–10^6	0	$10^{3.0}$–$10^{4.0}$
Sabin	1	$10^{5.2}$	0	$10^{3.0}$
	2	$10^{5.2}$	0	$>10^{1.4}$
	3	$10^{5.5}$	0	$10^{2.6}$

[1] $MPD_{50}(IC)$ = Monkey poliomyelitis $dose_{50}$ when the intracerebral route of inoculation is used
[2] $MPD_{50}(IS)$ = Monkey poliomyelitis $dose_{50}$ when the intraspinal route of inoculation is used

contains from 100 to 30,000 poliomyelitis doses for the monkey inoculated by the sensitive intraspinal route

CONCLUSIONS

The methods used in our study are not only more sensitive than those used by Cox and by Sabin, but they are also sufficiently discriminatory to distinguish strains of different degrees of neurovirulence Thus Sabin's Type 2 strain stands out from all the others as having the least amount of neurovirulence and the least tendency to spread in the spinal cord

Our results indicate that children have been fed poliovirus strains possessing greater neurotropic activity than had been suspected * The marked discrepancies between many of our titrations and those reported by Sabin and by Cox were surprising It was on the basis of their findings that the WHO Expert Committee on Poliomyelitis[2] in 1957 recommended that strains be used in field trials only if there was a "complete lack of paralytogenic activity on intracerebral inoculation of maximal doses (in excess of 10^7 TCD_{50}) in rhesus or cynomolgus monkeys and only minimal residual neurotropism by spinal inoculation in monkeys—i e, only rare development of localized, non progressive paralysis in monkeys receiving doses of 10^6 TCD_{50} or more " Our results with both Sabin's and Lederle's strains have since been confirmed by a second impartial laboratory, the Division of Biologics Standards, National Institutes of Health[1]

In criticizing our results, Sabin[4] implied that we produced so much non-specific damage in the monkeys that the inoculation procedure and the retention of the inoculum in the cord might have been responsible for the paralytic manifestations A series of control tests with normal tissue culture fluids, with an ECHO virus, or with the vaccine strains diluted beyond their paralytogenic endpoint failed to produce disease or histological responses that in any way could be confused with those of poliomyelitis

The situation may be compared to an iceberg If one uses an insensitive method, one only detects the peak of the iceberg, but if one uses a more sensitive method, one can determine how large a base the iceberg has Whether the base

* The same may be said for children fed Koprowski's strains, in view of the high degree of neurotropic activity found in these strains by Sabin[2] and by the Division of Biologics Standards[1]

TABLE 25 COMPARATIVE TITERS OF CANDIDATE STRAINS

Type	Strain	50% Doses per ml					Polio Lesion Scores[1]			
		Monkey Kidney Tissue Culture TCD_{50}	Intracerebral Neurovirulence Expressed as MPD_{50} (IC)[2]		Intraspinal Neurovirulence Expressed as MPD_{50} (IS)[2]		Intracerebral Inoculation		Intraspinal Inoculation	
			Clinical Disease[3]	Polio Lesions	Clinical Disease[3]	Polio Lesions	Cervical	Lumbar	Cervical	Lumbar
1	Lederle Sabin	$10^{7.8}$ $10^{7.3}$	$\propto 10^{3.0}$ $<10^{0}$	$>10^{3.6}$ $<10^{0}$	$10^{6.6}$ $10^{4.4}$	$10^{5.5}$ $10^{5.0}$	457 0	649 24	844 123	1,597 837
2	Lederle Sabin	$10^{8.9}$ $10^{7.3}$	$\propto 10^{3.0}$ Neg	$>10^{2.5}$ Neg	$10^{5.0}$ $10^{2.4}$	$10^{5.3}$ $>10^{3.4}$	487 0	704 0	1,009 44	1,778 346
3	Lederle Sabin	$10^{7.7}$ $10^{7.5}$	10^{0} Neg	10^{0} Neg	$10^{4.7}$ $10^{2.0}$	$10^{5.8}$ $10^{4.0}$	179 0	232 0	584 355	1,250 850

[1] Undiluted through 10^{-2} used for calculation of polio lesion scores for monkeys *inoculated intracerebrally*, and undiluted through 10^{-4} for those inoculated *intraspinally*

[2] MPD_{50} (IC) = monkey poliomyelitis dose$_{50}$, intracerebrally, i e , the dose which produced specific lesions in 50% of the intracerebrally inoculated monkeys. MPD_{50} (IS) = monkey poliomyelitis dose$_{50}$, intraspinally, i e , the dose which produced specific lesions in 50% of the intraspinally inoculated monkeys

[3] Paralysis or weakness due to multiplication of poliovirus

Monkey 357 showed weak legs immediately after inoculation, with progressive improvement to normal behavior by the 6th day.

Monkey 359 developed a paralyzed left leg immediately after the inoculation. Improvement in function was noted 24 hours later, and steadily progressed; by the 7th day the monkey appeared normal.

Monkey 354: slight perivascular cuffing was seen in white matter, or posterior horn, in cervical level 8 and thoracic levels 2 and 3.

Inoculation trauma in the monkeys inoculated intracerebrally usually involved arms, neck, or walking in a circle. In those inoculated intraspinally, the inoculation trauma was restricted to the legs.

→ This symbol indicates paralysis or weakness which persisted throughout the observation period.

← This symbol indicates that the animal recovered the affected function and appeared normal by the end of the observation period.

In the tests of Types 1 and 2, cynomolgus monkeys were used exclusively. In testing the higher dilutions of Type 3, a few rhesus monkeys were also used; their responses were not significantly different from those of the cynomolgus monkeys (see detailed Tables 10b and 12b).

Weights of all monkeys ranged from 2 to 6 pounds, with most animals weighing 3 or 4 pounds.

Scores of 3 (0/++) and less were considered negative for purposes of the summary tabulations.

Virus spread to cervical cord (arm involvement = A) and to medulla (tremors, ataxia, convulsions = M).

of neurovirulent material, present to varying degrees in all of the oral poliovirus vaccines currently being used in field trials, is at all dangerous remains to be determined

References

1 Murray, R, Kirschstein, R, Van Hoosier, G, Jr, and Baron, S Comparative virulence for Rhesus monkeys of poliovirus strains used for oral administration Proceedings, Conference on Live Poliovirus Vaccines, Document Number 25 In press

2 Sabin, A B: Present position of immunization against poliomyelitis with live virus vaccines Brit M. J *1*: 663-680 (March 14), 1959

3 World Health Organization: Expert Committee on Poliomyelitis, Second Report. WHO Technical Report Series No *145*: 1-83, 1958

FOOTNOTES TO TABLES

*The number shown in this column indicates day after inoculation on which designated sign was observed

...ralyzed left leg
... paralyzed left arm

CPL = completely paralyzed legs, CPRL = completely paralyzed right leg, CPLL = completely paralyzed left leg

CPA = completely paralyzed arms, CPRA = completely paralyzed right arm; CPLA = completely paralyzed left arm

For details of duration of paralysis or weakness, and of signs due to inoculation, see Tables 14 through 19

† Cord lesions

The code figures in parentheses indicate the range of severity (score) of the lesions observed in the cord

The number at the left of the parentheses is the sum of the score of 10 levels

The maximum score for both hemisections of one level is 8 (++++/++++)

Root lesions

− = No root lesions present

+ = Root lesions present anywhere above upper limits of needle injury

⊕ = Root lesions present, but only at or below spinal cord inoculation site

Key to Score for Each Hemisection

0 = No lesions in hemisection

+ = Single focal neuronal lesion (one or more nerve cells), or single vascular lesion, or single cellular infiltrate of monocytes

++ = Combination of two or three of above lesions

+++ = Clusters of neuronal lesions plus more extensive vascular lesions, and areas of cellular infiltration

++++ = Gross destruction of nerve cells, plus extensive areas of infiltration and perivascular cuffing

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CHART 1 PASSAGE HISTORY OF LEDERLE ATTENUATED POLIOVIRUS (SM STRAIN—TYPE 1)

Monkey Virulence Test				S+M Pool (MK-TC) ↓ Mice I 8 26 pas ↓		
Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Chick Embryo Tissue Cult	Times Plaqued in Monkey Kidney Cells
				10 pas →	14 pas → (5 alterations)	No 1
7 2	0/29	6 5	2/23	2 pas ↓		
6 2	0/8	5 5	2/7			
5 2	0/8	4 5	2/7	1 pas		
4 2	0/8	3 5	0/7	Pool N90 (7 5)* ↓		No 2
3 2	0/8	2 5	0/4	2 pas ↓		
7 2	0/4	6 5	0/4	Pool 19 (7 5)* ↓		No 3
7 9	0/4	7 2	0/1	2 pas ↓ Pool 21 (8 2)* ↓		No 4
—	—	6 7	1/5	2 pas ↓ Pool 42 (7 7)*		
5 4	0/10	4 7	0/5		1 pas Pool 45 (5 7)*	
6 9	0/10	6 2	0/10	↓ 1 pas		
—	—	5 2	3/10	Pool 400B 10 M3 (7 7)* (Seed virus)		No 5
6 2	1?/10	5 2	3/10	↓ 2 pas ↓		
5 2	0/10	4 2	4/10	Pool 7 1231-114 (7 5)* (Seed virus)		
4 2	0/9	3 2	4/10			

*Log TCD_{50} per ml

4. CUMULATIVE TESTING EXPERIENCE WITH CONSECUTIVE LOTS OF ORAL POLIOMYELITIS VACCINE

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DR. CABASSO *(presenting the paper)* Any vaccine proposed for general and continued use must first satisfy certain laboratory criteria of potency and safety of successive lots produced over a period of time. Oral poliomyelitis vaccine is no exception to this rule, although specific production and testing standards for it have not yet been established.

A number of safeguards are obvious prerequisites to the release of a given production lot for clinical trials. For the most part, these were suggested by experience with other vaccines also prepared in monkey kidney culture. They are particularly critical in the case of oral poliomyelitis vaccine, which can not contain a preservative that would control undesirable accompanying microorganisms. The measures taken must be so applied as to rule out, with a well-founded degree of certainty, the presence in the vaccine of any organism, harmful or not, other than poliovirus. They also must serve to ascertain that the avirulence of the vaccine virus remains unchanged during the course of repeated cultivation, and, under ideal conditions, they should permit measurement of the immunogenic potency of the vaccine batch under test.

With oral poliomyelitis vaccine only the first of these three requirements can be carried out in the laboratory under conditions reflecting reality. The other two questions, of vaccine safety and potency for man via the oral route, could perhaps be answered only by use of the chimpanzee in prohibitive and unrealistic numbers. Consequently, for the answer regarding avirulence, rather artificial and sometimes drastic methods of inoculation of certain species of monkeys such as the rhesus or the cynomolgus must be resorted to. And since in the case of oral poliomyelitis vaccine antigenic stimulation is a function of *in vivo* viral multiplication, determination of the virus content of the vaccine is a poor but, under the circumstances, necessary and logical substitute.

Although our laboratories have been engaged in the study of a live virus oral poliomyelitis vaccine since the late 1940's, this report will attempt to deal only with our experience since 1957 in the production and testing of serial lots of vaccine, some of which were used in small and large scale clinical trials. This experience will be correlated to the immune status of, and the clinical results obtained in, the populations fed, in an effort to determine to just what degree monkey tests can measure the safety of the vaccine virus for man.

Virus Strains

The origin and development of the Lederle strains of attenuated polioviruses have been amply described and summarized in previous reports from this laboratory and elsewhere.[1] A brief, up-to-date résumé follows.

Type 1 virus originated from a mixture of tissue culture fluids respectively infected with the Sickle and Mahoney Type 1 isolates (Chart 1).[2] The strain, derived from 26 serial intraspinal passages in mice, was subsequently transferred to monkey kidney tissue culture for 10 generations, was passed 14 times in chick embryo tissue culture, and was plaqued out on monkey kidney monolayers. Following five alternations between monkey kidney and chick embryo cul-

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CHART 1 PASSAGE HISTORY OF LEDERLE ATTENUATED POLIOVIRUS (SM STRAIN—TYPE 1)

8+M Pool (MK-TC)
↓
Mice 18 26 pas
↓

Monkey Virulence Test						
Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Chick Embryo Tissue Cult	Times Plaqued in Monkey Kidney Cells
				10 pas →	14 pas →	No 1
				2 pas	5 alterations	
				1 pas		
7 2	0/29	6 5	2/23			
6 2	0/8	5 5	2/7			
5 2	0/8	4 5	2/7	Pool N90 (7 5)*	→	No 2
4 2	0/8	3 5	0/7			
3 2	0/8	2 5	0/3			
				2 pas		
7 2	0/4	6 5	0/4	Pool 19 (7 5)*	→	No 3
				2 pas		
7 9	0/4	7 2	0/4	Pool 21 (8 2)*	→	No 4
				2 pas		
—	—	6 7	1 5	Pool 12 (7 7)*		
					1 pas	
5 4	0 10	4 7	0 5		Pool 45 (5 7)*	
				1 pas		
6 9	0 10	6 2	0 10			
—		5 2	[illegible] 10	Pool 100H 10 M1 (7 7)* (Seed virus)		No 5
				2 pas		
6 2	[illegible]/10	5 2	1 10			
5 2	0/10	4 2	1 10	Pool 7 123[illegible] 1[illegible] (7 5)* (Seed virus)		
4 2	0 4	3 2	1 10			

*Log TCD_{50} per ml

Chart 2 Passage History of Lederle Attenuated Poliovirus (MEF 1 Strain—Type 2)

Mouse Brain

↓

Suckling Hamsters Intracerebral (157 passages)

↓

Chick Embryo (17 passages)

Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio (Monkey Virulence Test)	Logs of Virus Inoculated (Monkey Virulence Test)	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Times Plaqued in Monkey Kidney Cells
				11 pas	→ No 1
				↓ 1 pas ↓	
7 5	0/5	6 5	4/5	1 pas (7 5)*	→ No 2
				↓ 2 pas ↓	
				1 pas	
0 2	0/9	5 2	2/8		
5 2	0/10	4 2	1/8	Pool 7-1232-200 (7 5)* (Seed virus)	
4 2	1/10	3 2	0/8		

*Log TCD_{50} per ml

	Monkey Virulence Test			Human Stool (Fox P1140)	
Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Times Plaqued in Monkey Kidney Cells
				3 pas	→ No 1
				1 pas*	
6.9	0/7	6.2	2/4	1 pas (7.2)*	→ No 2
7.7	1/14	7.0	1/8	2 pas	
6.7	0/10	6.0	1/4		
5.7	0/4	5.0	0/4	1 pas (8.0)*	→ No 3
4.7	0/4	4.0	0/4		
				1 pas	
6.4	0/5	5.7	0/5	1 pas (6.7)*	
7.2	0/14	6.5	0/4	1 pas	
6.2	0/4	5.5	0/4	1 pas (7.5)*	
5.2	1/4	4.5	0/4		→ No 4
4.2	0/4				
				2 pas	
7.5	0/5	6.5	0/5	1 pas	
6.5	0/4	5.5	2?/5	Pool 7 1233 310 (7.5)* (Seed virus)	→ No 5
7.2	1?/10	4.5	1/5	2 pas	
6.2	0/4	6.2	2 1?/10		
5.2	0/6	5.2	0/5	Pool 7 1233 310 I (7.2)* (Seed virus)	
		4.2	0/5		

*Log TCD_{50} per ml

tures, four additional plaquings were carried out before selection of the original seed virus designated Pool 400B-10 M3 A fifth plaquing, derived from that pool, was the source of a second seed pool (7-1231-114) Intracerebral and intraspinal inoculations of monkeys with plaque-derived virus pools repeatedly demonstrated at all passage levels the nearly complete loss of virulence of the virus when inoculated intracerebrally, and a considerable diminution of its intraspinal activity

Type 2 virus, as indicated in Chart 2, descended from the MEF1 strain following its intracerebral passage 157 times in suckling hamsters, its adaptation to, and propagation in, chick embryos for 17 consecutive generations, and its passage in monkey-kidney cultures[3] Seed virus pool 7-1232-200 was obtained after 11 transfers and two consecutive plaquings in this latter host cell The reason for growing the virus back in monkey-kidney-tissue culture instead of the chick embryo as the source of vaccine was the relatively low titers achieved in chick embryos which rarely approached $10^{5.0}$ mouse LD_{50} per ml of a 20-per-cent suspension

The original Type 3 strain was an essentially avirulent variant isolated in monkey-kidney-tissue culture by Dr John P Fox from the stool of a healthy carrier (Chart 3) A monkey-kidney culture pool infected with the progeny of the fourth plaquing of this strain constituted the original seed virus Pool 7-1233-310 A second seed pool (7-1233-310E5) was obtained with one additional plaquing from the first pool

Monkey virulence tests on both Type 2 and Type 3 viruses between the various plaquings yielded results essentially similar to those with the Type 1 strain

Production of Vaccine

This report concerns the production and testing of a total of 31 seed and vaccine lots of the three types of virus

The genealogy and distribution of Type 1 vaccines in various clinical trials are illustrated in Chart 4. It can be seen that seven vaccine batches originated from two seed lots, and the second seed virus was only one plaquing removed from the first. Four of these vaccines were fed in eight separate trials carried out in seven different countries In all but one of the trials, single lots of vaccine were used throughout each trial However in Uruguay, because of the large number of individuals vaccinated, vaccines from four different lots were employed.

The history of Type 2 vaccine production and clinical trials is shown in Chart 5 Here a single seed virus was the progenitor of all 10 vaccine lots produced, and four of these lots were used in the field studies

For Type 3, as indicated in Chart 6, production and trial histories of the vaccine are similar to those of Type 1 in that two closely related seed pools were used for the 10 lots produced Two of the lots were sufficiently large and of sufficient titers to satisfy the needs of all clinical trials undertaken thus far.

All of the vaccine lots were produced in monkey-kidney tissue Povitsky bottle and tube cultures were prepared according to the method of Dulbecco and Vogt, using multiple trypsinizations at room temperature or the overnight technique at 4°C suggested by Bodian[4] Growth fluid consisted of Earle's basal medium to which were added Eagle's vitamin mixture, 0 5% lactalbumin hydrolysate and 2% calf serum[5] Sodium bicarbonate content of the fluid was 0 11% The growth medium was dispensed at the rate of 100 ml per Povitsky bottle of 2,000-ml capacity, or 1 ml per tube. The various lots of calf serum employed were routinely tested for poliovirus "neutralizing substances" before their addition to the growth medium, and those with titers higher than 1 8 were discarded

Monolayers were allowed to grow at 37°C for 6 days before introduction of the virus Since experimental evidence had shown that cultures which were "leached out" with renewal fluid before inoculation did not yield substantially higher titers than those where leaching was omitted inoculation with virus was routinely carried out after simple removal of the growth medium and its replacement with renewal solution in amounts of 500 ml per Povitsky bottle and 1 ml per tube

The renewal fluid also was made up of Earle's basal solution except that the sodium bicarbonate concentration of 0 22% was retained and the serum was omitted In addition it contained the following substances: 0 1% yeast extract 0 1% bovine albumin, 0 03% 1 glutamine and 0 5% lactalbumin hydrolysate Its pH was between 7 4 and 7 6 Antibiotics were added in the following concentrations per ml. of either

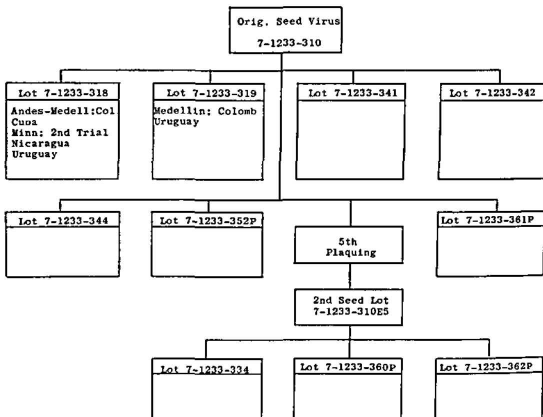
CHART 4 PRODUCTION SUMMARY OF TYPE 1 ORAL POLIOMYELITIS VACCINE

- Orig. Seed Virus 400B-10-M3
  - Lot 7-1231-110: Minn 2nd Trial; Uruguay
  - 5th Plaquing
    - 2nd Seed Lot 7-1231-114
      - Lot 7-1231-121: Medellin: Colomb.; Haiti; Nicaragua; Uruguay
      - Lot 7-1231-126
      - Lot 7-1231-140
      - Lot 7-1231-162P
  - Lot 7-1231-115: Andes: Colombia; Costa Rica; Uruguay
  - Lot 7-1231-120: Cuba; Uruguay

CHART 5 PRODUCTION SUMMARY OF TYPE 2 ORAL POLIOMYELITIS VACCINE

- Orig. Seed Virus 7-1232-200
  - Lot 7-1232-213: Andes Colombia; Minn 2nd trial; Uruguay
  - Lot 7-1232-216: Medellin, Colomb.; Nicaragua; Uruguay
  - Lot 7-1232-217A: Medellin Colomb.
  - Lot 7-1232-222
  - Lot 7-1232-227: Costa Rica; Cuba; Haiti
  - Lot 7-1232-228
  - Lot 7-1232-240
  - Lot 7-1232-241
  - Lot 7-1232-242
  - Lot 7-1232-243

CHART 6. PRODUCTION SUMMARY OF TYPE 3 ORAL POLIOMYELITIS VACCINE



growth or renewal medium 50 units penicillin, 50 micrograms streptomycin, 25 micrograms neomycin and 50 units mycostatin.

As an additional safety precaution each pair of monkey kidneys was processed separately and the bottles derived from it provided individual vaccine harvests In most instances not less than 1 ml of undiluted seed virus was used for inoculation of the culture bottles. These were incubated at 37°C. and examined daily for progress of viral effect. Bottles showing the slightest evidence of bacterial or fungal contamination were immediately discarded The others were harvested on the third or fourth day, when the monolayer had completely detached from the glass. As already mentioned, all cultures from one monkey were harvested and pooled separately, and were given an identifying pool number. The pH of each pool was determined and if necessary it was adjusted back to 7.0 with M/2 monobasic sodium phosphate solution The pool was then filtered through a Hormon D9 asbestos pad and held overnight at 4°C. On the following day the pH was rechecked, and readjusted if necessary Samples for the various *in vitro* tests were removed, and the bulk of the pool was stored in a —20°C freezer

To ensure ready identification and to minimize errors, especially when working on a fairly large scale, samples and bulk harvests not only were marked with the virus type and pool number, but this information was written on large labels of different bright colors assigned from the start to the three types of virus red for Type 1, yellow for Type 2 and green for Type 3 These identifying colors followed the respective virus types to the final containers in which they were shipped

Testing of the vaccine was carried out in two steps. First, the undiluted individual monkey harvests were subjected to a battery of *in vitro* tests for the purpose of ruling out the presence of bacterial or fungal contaminants and of simian and measles viruses In addition, viral titration

TABLE 1. IN VITRO TESTS ON INDIVIDUAL MONKEY HARVESTS

(3- to 10-liter lots)

PURPOSE OF TEST	TEST SYSTEM	OBSERVATION (DAYS)
To rule out		
1 Bacterial contaminants	Thioglycolate Broth—Room temperature (22°-24°C) and 37°C	14
2 Fungal contaminants	Sabouraud's Broth—Room temperature (22°-24°C) and 37°C	14
3 Simian viruses	a) Vaccine-Homologous Antiserum mixture in MK tubes	14
	b) Passage of fluid from *a* in second set of MK tubes on 7th day	7
4 Measles virus	Vaccine-Homologous Antiserum mixture in HeLa cultures	14
To determine		
1 Polio virus titer	10-fold dilutions in MK tubes—12 tubes per dilution	8
2 Polio virus identity	Mixtures of vaccine and antisera to 3 types of virus in MK cultures	8

and identification tests were performed When the results of all these tests were considered satisfactory, testing was continued by *in vivo* methods, in some cases on the individual monkey harvests, but more frequently on 16- to 20 liter vaccine lots made by pooling two to four individual harvests

The second series of tests was designed to rule out the presence of CNS-Herpes, Coxsackie and B viruses, as well as the tubercle bacillus Finally the neurovirulence test was performed in cynomolgus monkeys

Upon completion of all tests, satisfactory vaccine lots were diluted to the desired virus concentration in one of two ways- either by absorption on granulated gelatin to obtain a relatively dry, free-flowing preparation which could be filled into hard shell gelatin capsules, or by mixing the fluid with a flavored and stabilizing syrupy excipient

Testing of the Vaccine

The *in vitro* testing schedule for individual monkey harvests is summarized in Table 1 In brief, each preparation is tested for bacterial sterility in thioglycolate broth, at both room temperature (22°-24°C) and 37°C A similar test for fungi is performed in Sabouraud's broth

Testing for simian viruses is carried out with specially prepared antisera When using monkey-kidney-propagated poliovirus for the hyperimmunization of laboratory animals, it is difficult to exclude the possibility of a concomitant simian viral agent, and antibodies against it may be elicited simultaneously with those produced against poliovirus Similarly, monkeys are unsuitable for preparation of the antisera to be used in this test, since they may already have simian viral antibodies from naturally acquired infections Consequently we have adopted the following routine procedure The three types

of poliovirus are "purified" by at least three, and in some cases up to eight, terminal dilution passages in HeLa cultures. These latter are not known to be susceptible to most simian viruses, and therefore should yield poliovirus pools reasonably free from other contaminating viruses. Such pools were used in adjuvant mixtures for the hyperimmunization of donor rabbits or chickens.

The simian virus exclusion test is performed by mixing the undiluted vaccine sample with an equal volume of undiluted homologous antiserum. Following three hours of incubation at room temperature, the virus-serum mixture is inoculated into 10 monkey-kidney-culture tubes in amounts of 0.2 ml. per tube. These are incubated at 37°C. and examined daily for cytopathology. At the end of seven days the fluid is removed from these tubes, pooled and reinoculated into 10 additional, freshly prepared, monkey-kidney-culture tubes, while the original set of tubes is renewed with fresh medium. Five to 10 per cent homologous antiserum is added to the 20 tubes, which then are returned to the incubator at 37°C. and observed for another seven-day period. When, occasionally, an apparent virus break-through occurs, the resulting agent is grown out and resubmitted to the same procedure. It should be added that throughout this set of tests proper controls are always included.

For the exclusion of measles virus, the initial virus-homologous antiserum mixture referred to above is also inoculated into 10 HeLa-culture tubes. These are incubated at 37°C. for 14 days and observed for the appearance of any multinucleated giant cells.

Both titration and identification of the virus are made in tubes of monkey-kidney cultures after eight days of incubation at 37°C. For titration, 10-fold dilutions are used, with 0.1 ml. inocula and 12 tubes per dilution. Titers are

TABLE 2 IN VIVO AND OTHER TESTS ON POOLS OF 2 TO 4 INDIVIDUAL MONKEY HARVESTS

(16- to 20-liter lots)

PURPOSE OF TEST	TEST SYSTEM	OBSERVATION (DAYS)
To rule out		
1 CNS-Herpes viruses	Adult mice (12)—I.C. and I.P.	21
2 Coxsackie viruses	Newborn mice (3 litters)—I.C. and I.P.	21
3 B virus	Rabbits (3)—I.C., I.P. and 4 x I.D.	21
4 T.B.	a) Guinea-pigs (3)—I.C. and I.P.	42
	b) Lowenstein-Jensen medium	42
To determine		
Monkey neurovirulence*	a) I.C.—Undil. –1.0, $10^{-1.6}$, $10^{-2.6}$ (0.5 ml. bilaterally)	28
	b) I.S.—Undil. –1.0, $10^{-1.6}$, $10^{-2.6}$ (0.1 ml.)	21
	c) Histopathological examination of cervical and lumbar enlargements of all monkeys	

* All tests performed in cynomolgus monkeys

calculated by the Reed and Muench formula* Identification, the final *in vitro* test, employs mixtures of vaccine and antisera to the three types of virus

Vaccine lots which pass all of the *in vitro* tests are then subjected to the tests indicated in Table 2

Absence of CNS and herpes viruses is determined by inoculating each of 12 adult mice with 0 03 ml. of undiluted vaccine introcerebrally and 0 1 ml intraperitoneally The mice should show no evidence of central nervous system disturbance during a period of 21 days To rule out the presence of Coxsackie viruses, at least three litters of suckling mice are used Each animal receives 0 01 ml of undiluted vaccine intracerebrally and 0 1 ml intraperitoneally, and should remain normal for 21 days It should be noted that, as the MEF1 Type 2 strain used in this laboratory still possesses its mouse marker, the CNS-herpes and Coxsackie exclusion tests have to be carried out in the presence of homologous antiserum

For the detection of B virus, three 4- to 6-lb rabbits are each given 0 25 ml of the bulk vaccine intracerebrally, 9 0 ml subcutaneously, and four intradermal injections of 0 25 ml each, administered at four different sites These ani-

TABLE 3 INTRACEREBRAL TEST WITH TYPE 1 VACCINE LOTS MONKEYS DEAD BEFORE END OF OBSERVATION PERIOD

Lot No	No of Monkeys Tested	Dead Before 28 Days		Ratio		
		No of Monkeys	Time of Death (In Days)	Paralytic	Polio Histopathological*	
					Cervical	Lumbar
110	25	1 1 2	1–5 6–10 11–20	0/1 0/1 0/2	0/1 0/1 0/2	0/1 0/1 0/2
114	30	1	11–20	0/1	0/1	0/1
115	30	0	—	—	—	—
120	30	0	—	—	—	—
121	30	1	6–10	0/1	0/1	0/1
126	22	1 1	11–20 21–28	0/1 0/1	0/1 0/1	0/1 0/1
140	22	0	—	—	—	—
162P†	22	4 5	6–10 11–20	0/4 1?/5	0/4 1+1?/5	1/4 2+1?/5
TOTALS	211	17	—	1?/17	1+1?/17	3+1?/17

PERCENT DEAD 8 0

* All histopathological reactions were of the inflammatory type; no neuronal loss or damage was observed
† Scored histopathologically by method B All others scored by method A

TABLE 4 INTRACEREBRAL TEST WITH TYPE 2 VACCINE LOTS: MONKEYS DEAD BEFORE END OF OBSERVATION PERIOD

Lot No	No of Monkeys Tested	Dead Before 28 Days		Ratio		
		No of Monkeys	Time of Death (In Days)	Paralytic	Polio Histopathological*	
					Cervical	Lumbar
200	30	1 1	6–10 11–20	0/1 1?/1	0/1 0/1	0/1 0/1
213	30	1 3	1–5 11–20	0/1 0/3	1/1 2/2	1?/1 2/2
216	60	4 1	6–10 11–20	1?/4 1/1	1/3 0/1	2/3 0/1
217A	40	1 1 3	1–5 6–10 11–20	0/1 0/1 0/3	0/1 0/1 1/3	0/1 0/1 1/3
222	22	1	1–5	0/1	0/1	0/1
227	22	1	6–10	0/1	1/1	1/1
228	22	0	—	—	—	—
240†	22	1 3 1	1–5 11–20 21–28	0/1 0/3 0/1	0/1 0/3 0/1	0/1 0/3 0/1
241†	22	2 2 2 2	1–5 6–10 11–20 21–28	0/2 0/2 1/2 0/2	0/2 0/2 1/2 0/2	0/2 0/2 1/2 0/2
242†	22	1 1 2 1	1–5 6–10 11–20 21–28	0/1 0/1 0/2 0/1	0/1 0/1 1?/2 0/1	0/1 1?/1 1/2 1?/1
243†	22	3	11–20	0/3	0/3	0/3
TOTALS	314	39	—	2+2?/39	7+1?/37	8+3?/37

PERCENT DEAD 12 4

* All histopathological reactions were of the inflammatory type; no neuronal loss or damage was observed
† Scored histopathologically by method B All others scored by method A

mals also are observed for 21 days, during which time they should be free of paralysis and of necrosis at the sites of inoculation

One TB test consists of inoculating three 300- to 500-gram guinea pigs with 0.2 ml. of undiluted vaccine intracerebrally and 1 0 ml intraperitoneally. The animals should show no clinical evidence of disease during a 42-day observation period. They then are sacrificed and examined for gross pathological signs of TB, and smears are made from the meninges under the medulla to be examined for acid-fast organisms In addition to the *in vivo* test, tubes of Lowenstein-Jensen medium are each inoculated with 0 5 ml of vaccine and incubated for 42 days at 37°C

The series of *in vivo* tests is completed with the checking of the vaccine for neurovirulence in cynomolgus monkeys. The animals that have been used weighed between 2.5 and 5 lbs and were received directly from the Phillippine Islands During the past year their average size was somewhat smaller than is desirable, and some groups reached the laboratory in rather poor condition Despite intensive conditioning care soon after their arrival, an appreciable number was lost before inoculation, during the first two to three weeks, in some groups this number amounted to 20 per cent of the total All monkeys were tuberculin tested on arrival and were not used for testing for at least three weeks thereafter The neurovirulence test was carried out by both the intracerebral and the intraspinal route

Intracerebral Monkey Inoculation

Of the vaccine pools reported on here, all Type 1 lots (8 in number), 7 of the 11 Type 2 lots, and 3 of the 11 Type 3 lots were inoculated into the thalamic region in 0 5 ml amounts on each side Four type 2 and four Type 3 lots were inoculated in 0 5 ml. amounts in only one site, and the remaining four Type 3 pools were also injected in one site, but in amounts of 1.0 ml The extent of traumatic loss of animals varied but little when using these various modes of inoculation, and rarely exceeded an occasional monkey out of groups of 22 to 30

At different times during the course of the observation period a number of test monkeys were found dead, most of them without having shown any paralysis and a few with a slight weakness or paralysis of one limb In most instances autopsy revealed pneumonia or extremely heavy worm infestation or both In any event, the spinal cords of all these animals were sectioned for histopathological studies, as were those of all survivors on the 28th day of observation

That most of these early deaths were in no way related to poliomyelitis is borne out by the histopathological results summarized in Tables 3, 4, and 5.

Table 3 indicates that of 211 monkeys inoculated intracerebrally with Type I vaccines, 17 or 8 0 per cent died before the end of the observation period. Only one death, which might be considered traumatic, occurred within five days of inoculation, all the others occurring after the sixth day and up to the 28th day. Only one of the 17 monkeys had shown any, and that a questionable, sign of paralysis of one limb before death, and 13 were histologically negative for poliomyelitis Of the four positive monkeys, none *had any neuronal destruction, and all reactions* observed were of the inflammatory type

Table 4 shows that of 314 monkeys inoculated intracerebrally with Type 2 vaccine, 39 or 12.4 per cent died before the end of the observation period Only seven of these deaths occurred during the first five days and can be considered traumatic Of the 32 animals which died after the sixth day, two were suspected of some degree of paralysis of one limb before death, and two of only weakness of one limb On histological examination 11 animals showed some neuronal loss or inflammation, the others were completely negative However, as will be indicated later, neuronal damage in the positive animals was of the same degree as that found in surviving, nonparalyzed animals inoculated with some lots of Type 2 vaccine

Table 5 summarizes the early deaths of monkeys inoculated with Type 3 vaccine. Here, 17 of 246 animals, or 6 9 per cent, died before the 28th day, three of them within the first five days In one of these three, weakness of one limb was observed on the day following inoculation, and as suggested by a completely negative histopathology of the cord, must have been due to trauma. The histopathological reactions observed in three of the animals were of the inflammatory type, and no neuronal loss or damage was noted

All vaccine lots were inoculated intracerebrally both in undiluted form and as 10^{-1} and 10^{-2}

TABLE 4 INTRACEREBRAL TEST WITH TYPE 2 VACCINE LOTS: MONKEYS DEAD BEFORE END OF OBSERVATION PERIOD

LOT No	No OF MONKEYS TESTED	DEAD BEFORE 28 DAYS		RATIO		
		No OF MONKEYS	TIME OF DEATH (IN DAYS)	PARALYTIC	POLIO HISTOPATHOLOGICAL*	
					CERVICAL	LUMBAR
200	30	1 1	6–10 11–20	0/1 1?/1	0/1 0/1	0/1 0/1
213	30	1 3	1–5 11–20	0/1 0/3	1/1 2/2	1?/1 2/2
216	60	4 1	6–10 11–20	1?/4 1/1	1/3 0/1	2/3 0/1
217A	40	1 1 3	1–5 6–10 11–20	0/1 0/1 0/3	0/1 0/1 1/3	0/1 0/1 1/3
222	22	1	1–5	0/1	0/1	0/1
227	22	1	6–10	0/1	1/1	1/1
228	22	0	——	—	—	—
240†	22	1 3 1	1–5 11–20 21–28	0/1 0/3 0/1	0/1 0/3 0/1	0/1 0/3 0/1
241†	22	2 2 2 2	1–5 6–10 11–20 21–28	0/2 0/2 1/2 0/2	0/2 0/2 1/2 0/2	0/2 0/2 1/2 0/2
242†	22	1 1 2 1	1–5 6–10 11–20 21–28	0/1 0/1 0/2 0/1	0/1 0/1 1?/2 0/1	0/1 1?/1 1/2 1?/1
243†	22	3	11–20	0/3	0/3	0/3
TOTALS	314	39	——	2+2?/39	7+1?/37	8+3?/37

PERCENT DEAD 12 4

* All histopathological reactions were of the inflammatory type, no neuronal loss or damage was observed

† Scored histopathologically by method B All others scored by method A

Regardless of the gauge of the needle, as in the intracerebral test with early batches of vaccine, 0.1 ml of undiluted tissue culture fluid and of 10^{-1} and 10^{-2} dilutations was injected into the lumbar enlargement of groups of ten animals per virus dilution. More recent lots of vaccine have been injected into ten, six and six monkeys per undiluted, 10^{-1} and 10^{-2} dilutions, respectively

A question has lately been raised as to whether in the early days of our work with this technique the fourth intervertebral space above the iliac crest was always the site of the inoculation. Review of histopathological specimens indicated that the correct site apparently was hit in at least 71 per cent of the cases and that in about 5 per cent the needle track was in the white instead of the gray matter; no needle track could be seen in the rest (24 per cent). Evidence also was obtained that in a few cases inoculation was into the fifth intervertebral space. However, of late both the iliac crest and the level of the last ribs have been used as landmarks for location of the inoculation site, and the record of properly located inoculations has been considerably improved. Other details pertaining to this procedure of intraspinal inoculation of monkeys have recently been described in detail by Dr Sabin[7]. It should be added that, regardless of the care with which this technique is applied the rate of traumatic paralysis is always greater than that following intracerebral inoculation

Beginning 24 hours following inoculation and daily thereafter, inoculated monkeys are exercised to observe any deviation from normal gait and comportment. Signs of weakness or paralysis on the very first day are considered to have been caused by trauma, unless on subsequent days evident progress of the paralysis is observed. Cords of animals dead of undetermined causes during the observation period are saved for histopathology regardless of whether paralysis was present prior to death. Animals showing advanced degrees of weakness or paralysis before termination of the test are sacrificed, examined *post mortem*, and their cords are removed for histopathology. The test is terminated on the 21st day, when all survivors are sacrificed and subjected to gross pathological examination; all cords are placed in 10 per cent formalin, and processed for microscopic histopathological examination

Neuro-Histopathology

Two methods were used for histological study. With Method A, fixed cervical and lumbar enlargements were cut into 1 cm blocks. These were embedded in celloidin and cut serially into sections 12 microns thick. Every 20th section was stained by Nissl's method and examined. Scoring was essentially based on the estimated percentage of nerve cells lost from the anterior horns and was as follows: 0=no cell loss and no inflammatory reaction, ± = no significant cell loss but some inflammatory reactions such as perivascular infiltration with or without glial nodules, + = 10-20 per cent cell loss*, ++ = 21-40 per cent cell loss, +++ = 41-60 per cent cell loss, ++++ = 61-80 per cent cell loss and +++++ = above 80 per cent cell loss

Method B which has been adopted in our laboratory only recently, follows the directions described by Sabin and by Melnick[7]. Scoring by this method is as follows: 0=no lesions, + = single focal neuronal lession, single vascular lesion or single area of cellular infiltration, ++ = combination of two or three of the above lesions, +++ = cluster of neuronal lesions plus more extensive vascular lesions and areas of cellular infiltration, ++++ = gross destruction of nerve cells plus extensive areas of infiltration and perivascular cuffing. In addition this method recommends that if no needle track can be discerned in the anterior horn of the lumbar cord of a monkey injected intraspinally, this animal not be considered in the final tally of results.

Test Results

Freedom from contaminating bacteria, molds and viruses was general for all tested vaccine lots. In very few cases a pseudomonas organism of the fluorescent group was recovered from individual monkey harvests, but this was readily cleared up by an additional filtration before submission of the lot to other tests. In no instance were tubercle bacilli or CNS, herpes, Coxsackie or B viruses encountered. Similarly except for the so-called "foamy agent," which also was seen quite consistently in control cultures, no recognizable simian viruses were isolated, despite the very large number of tests

* Cell loss below 10 per cent was considered to be within experimental error

TABLE 5 INTRACEREBRAL TEST WITH TYPE 3 VACCINE LOTS: MONKEYS DEAD BEFORE END OF OBSERVATION PERIOD

Lot No	No of Monkeys Tested	Dead Before 28 Days: No of Monkeys	Dead Before 28 Days: Time of Death (In Days)	Ratio: Paralytic	Ratio: Polio Histopathological*: Cervical	Ratio: Polio Histopathological*: Lumbar
310†	10	1	11–20	0/1	0/1	0/1
318†	30	2 2	1–5 11–20	0/2 0/2	0/2 0/2	0/2 0/2
319†	30	0	—	—	—	—
334	22	0	—	—	—	—
341	22	0	—	—	—	—
342	22	2	11–20	0/2	0/2	0/2
344	22	1	11–20	0/1	0/1	0/1
352P	22	1	1–5	1?/1	1/1	0/1
360P	22	2 1 1	1–5 6–10 11–20	1?/2 0/1 0/1	0/2 0/1 1/1	0/2 0/1 1/1
361P	22	1	6–10	0/1	0/1	0/1
362P	22	1 2	6–10 21–28	0/1 0/2	0/1 1?/2	0/1 0/2
TOTALS	246	17	—	2?/17	2+1?/17	1/17

PERCENT DEAD 6 9

* All histopathological reactions were of the inflammatory type, no neuronal loss or damage was observed
† Scored histopathologically by method A All others scored by method B

dilutions Early batches were administered to 10 monkeys per dilution, and some have had repeat tests The more recent routine has consisted of inoculating the undiluted vaccine into ten monkeys, and the 10^{-1} and 10^{-2} dilutions into groups of six animals each Inoculated monkeys were closely examined daily for 28 days for signs of limb weakness or paralysis, by exercising them in their runs. Any suggestive clinical signs were carefully recorded.

Intraspinal Monkey Inoculation

Most vaccine lots included in this report were inoculated intraspinally by means of a 26-gauge needle These tests were carried out before the recent studies which revealed the influence of the needle gauge on the outcome of the test.[7] Since we obtained this information, the new vaccine lots tested have been inoculated with a 20-gauge needle, although in some instances 20- and 27-gauge needle techniques were compared.

TABLE 6 VOLUME AND TITERS OF SUCCESSIVE LOTS OF TYPE 1 ORAL POLIO VACCINE

LOT NO	DATE PREPARED	VOLUME (ML.)	TITER, LOG TCD_{50} PER ML UNDIL TCF
7-1231-110	4- 5-57	5,000	7 7
114	6- 9-57	2,000	7 5
115	9- 4-57	8,000	7 3
120	9-25-57	15,000	7 7
121	3- 7-58	20,000	8 2
126	7-11-58	27,000	7 5
140	10-10-58	21,300	7 7
162P	3-25-59	15,000	7 3
	Total	113,300	

TABLE 7. VOLUME AND TITERS OF SUCCESSIVE LOTS OF TYPE 2 ORAL POLIO VACCINE

LOT NO	DATE PREPARED	VOLUME (ML)	TITER LOG TCD_{50} PER ML UNDIL TCF
7-1232-200	6-15-57	2,000	7 5
213	9- 6-57	18,000	7 5
216	1-31-58	20,000	7 5
217A	3- 3-58	16,000	6 8
222	9-18-58	22,000	7 3
227	10-10-58	13,000	7 3
228	10-10-58	13,000	7 0
240	1-12-59	3,500	7 3
241	1-12-59	5,000	7 6
242	1-12-59	4,000	7 4
243	1-12-59	3,500	7 4
	Total	120,000	

1 5 per cent for Type 1 vaccines However, of the 11 affected monkeys, three showed no histopathological lesion whatever, in either the cervical or the lumbar enlargement, and the others showed no greater histological changes than did clinically non affected animals Histopathological findings in non-paralyzed monkeys also were more frequent for early than for more recent pools of vaccine, and varied in extent among the different lots This is illustrated in Table 12, which presents the poliomyelitis histopathological picture of five representative Type 2 vaccine lots

The lot listed at the bottom of the table is almost completely negative, with but one of the animals showing any neuronal damage in the cervical area, and two in the lumbar This lot is quite typical of the more recently prepared vaccines, all of which give a greatly reduced neuronal-loss score following intracerebral inoculation The discrepancy between results with these and with earlier batches may be due to the fact that some of the individual monkey harvests included in early batches of vaccine were prepared by inoculating culture bottles with diluted seed virus, whereas only undiluted seed has been used during the past year

Effectiveness of the simian-virus-exclusion test system employed was checked in two ways First, samples of a Type 3 vaccine pool were deliberately contaminated with decreasing amounts of SV_{15}* The mixtures were then submitted to the routine exclusion test, in the presence of Type 3 antiserum, alongside an uncontaminated sample of the same Type 3 pool Within the 14-day observation period SV_{15} had been recovered from *every mixture expected to contain it, while the* uncontaminated sample gave no evidence of virus growth. In a second set of experiments the hyperimmune sera used in the routine test were assayed for neutralizing action on at least six known isolates of simian viruses SV_2, SV_4, SV_6, SV_{15}, SV_{28} and SV_{38} In every instance simian virus titers were of the same order, whether in the presence of normal or of poliomyelitis hyperimmune chicken sera This study is being extended to other known simian virus strains with both chicken and rabbit poliomyelitis antisera

Details pertaining to the date of preparation volume and titers of Type 1 vaccine pools are *given in Table 6 The period covered is two* years, during which time over 113 liters of vaccine were produced and completely tested TCD_{50} titers ranged from $10^{7.3}$ to $10^{9.2}$ per ml At the rate of $10^{6.0}$ TCD_{50} units per vaccination, the total volume produced should be sufficient for 4½ million doses.

Similar details are summarized in Table 7 for Type 2 vaccines The 11 lots amounted to 120 liters, ranging in titer from $10^{6.8}$ to $10^{7.9}$ TCD_{50} per ml Because of the somewhat lower average titer, this quantity of vaccine. although slightly greater than for Type 1, would yield approximately 2,600,000 clinical doses.

A summary of Type 3 vaccine lots is shown in Table 8 The total volume produced is 143 liters of an average titer of $10^{7.5}$ TCD_{50} per ml., also amounting to about 4½ million immunizations

Results of the intracerebral inoculation of monkeys *with the* 8 lots of Type 1 vaccine are shown in Table 9 It can be seen that only 3 of the 195 inoculated monkeys still alive on the 28th day of observation showed either a frank paralysis or a questionable involvement of a single limb. *These reactions occurred between* the 4th and 6th days following inoculation and progressed no further. Moreover, the three animals were negative for histopathological changes in their cervical and lumbar enlargements In fact, out of the 195 monkeys, neuronal damage was observed only in scattered animals, none of which showed paralysis. The damage ranged from a single focus of inflammation without cellular loss (± or questionable on the *table) to not more than 2+ (equivalent to 20* to 40 per cent cellular loss) In all, 10 animals, or 4 8 per cent, showed such changes in the cervical and 15, or 7 2 per cent, in the lumbar enlargement.

Table 10 summarizes results of the intraspinal monkey test with the same lots of Type 1 vaccine The number of animals showing paralysis is undoubtedly far greater than with intracerebral inoculation With undiluted vaccines 37/104 animals were affected (35 5%), with 10^1 dilutions, 35/73 (47 9%), and with 10^2 dilutions, 24/83 (28 8%). Most cases were limited to the lower extremities, and ranged *from weakness of a single limb to complete* paralysis of both legs Death of an affected animal was exceptional The great majority (260/272) survived the 21-day observation period and were sacrificed for histopathology Understandably, monkeys inoculated intraspinally showed marked histopathological changes Cellular loss and inflammatory reactions ranged from absent or minimal to total The rate of change was greater in the lumbar enlargement the site of inoculation, than in the cervical, averaging for the former 65 5 per cent of the animals and for the latter 42 0 per cent Similarly, the change tended to be greater in one anterior horn than the other, particularly in the cervical region It may also be noted that while 65.5 per cent of the animals had some degree of histopathology, the number showing clinical signs was of the order of 37 0 per cent, indicating that neuronal loss was not always accompanied by clinical signs

Of the 31 vaccine lots representing the three types of virus, that are reported on here, some early batches of Type 2 vaccine exhibited, on the whole, the greatest intracerebral activity As shown in Table 11, of 279 monkeys that either survived the observation period or showed some paralysis before death, 11 or 3 9 per cent had weakness or paralysis of a single limb, as against

* The authors are indebted to Drs. Samuel Bozeman of Pitman-Moore Company and Robert Hull of Eli Lilly Company for the various isolates of simian viruses received from them

TABLE 9 INTRACEREBRAL MONKEY VIRULENCE OF SUCCESSIVE LOTS OF TYPE 1 ORAL POLIO VACCINE, AT DIFFERENT DILUTIONS

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
110	7.7	0/3	0/9	0/9	0/5	0/9	0/10	1?/4	0/9	0/10
114	7.5	1?/10	0/10	0/9	1?/10	1?/10	0/9	1/10	1/10	0/9
115	7.3	0/10	0/10	1/10	0/10	0/10	1?/10	1?/10	0/10	1?/10
120	7.7	0/10	0/10	0/10	1/10	1/10	0/9	1/10	1/10	0/9
121	8.2	0/9	0/10	0/10	0/9	0/10	0/10	0/9	0/10	0/10
126	7.5	0/10	0/4	0/6	0/10	1/6	0/6	0/10	1/6	0/6
140	7.7	0/10	0/6	0/6	1/9	0/6	0/6	0/10	0/6	0/6
162P†	7.3	0/6	0/4	1?/4	0/10	1+1?/6	1?/6	1?/10	2+1?/6	2+1?/6
Totals		1?/68	0/63	1+1?/64	2+1?/73	3+2?/67	2?/66	2+3?/73	5+1?/67	2+2?/66
Percent		1.5	0	3.1	4.1	7.4	3.0	6.8	9.0	6.0
Percent of Total			1.5			4.8			7.2	

* Log TCD_{50} per ml

† Scored histopathologically by method B. All others scored by method A

TABLE 8. VOLUME AND TITERS OF SUCCESSIVE LOTS OF TYPE 3 ORAL POLIO VACCINE

LOT NO	DATE PREPARED	VOLUME (ML)	TITER: LOG TCD_{50} PER ML UNDIL TCF
7-1233-310	2-15-57	3,000	7 5
318	9-26-57	10,000	7 7
319	11-23-57	22,000	7 5
334	10-10-58	27,000	6 8
341	12- 4-58	4,000	8 0
342	12- 4-58	4,500	7 6
344	12- 4-58	5,000	7 7
352P	12-17-58	16,000	7 2
360P	12-17-58	16,500	7 1
361P	2-13-59	16,500	7 4
362P	2-17-59	18,500	7 6
	Total	143,000	

Each of the four other lots shown on the table was fed to at least 80,000 persons, and one, Lot 213, was fed to almost 200,000 As can be seen, the cervical and lumbar activity of these lots in monkeys seldom reached a 40-60 per cent cell loss (2 to 3 5 per cent of the cases), and in over 60 per cent of the animals findings were either completely negative or minimal (less than 10 per cent loss) Despite the relatively greater neuronal activity of these lots, not a single case of confirmed disease was reported as a result of their ingestion by human beings

It also is of interest that, as shown in Table 13, early lots of Type 2 vaccine, although somewhat more active intracerebrally, induced no more paralysis and produced no greater histopathological reactions by the intraspinal route than did Type 1 lots It may be recalled that for animals showing clinical paralysis or limb weakness following Type 1 inoculations, the rates were 35 5 per cent for the undiluted, 47 9 per cent for the 10^{-1} and 28 8 per cent for the 10^{-2} groups, or an over-all rate of 37 0 per cent For Type 2 the corresponding rates were 45 per cent, 41 per cent and 23 per cent, giving an average rate of 37.6 per cent. Type 2 vaccines were inoculated intraspinally into a total of 280 monkeys, of which 258 (92.1 per cent) survived the observation period.

Intracerebrally inoculated Type 3 vaccine lots behaved in monkeys very similarly to Type 1, as indicated in Table 14 Only 1.2 per cent of the inoculated animals (3 out of the 230 surviving the observation period) showed some weakness of one limb, and all three were histopathologically negative Histological changes as a whole were minimal, and consisted mostly of inflammatory reactions with little or no neuronal loss

Intraspinal paralytic rates of Type 3 lots, as shown in Table 15, were 26 7 per cent for the undiluted group and 36 per cent and 31 3 per cent for the 10^{-1} and 10^{-2} dilutions respectively or an average of 30 7 per cent of the 228 out of 239 surviving animals The histological damage produced by this route of infection was not very different for Type 3 from that for Type 1 virus

Considerations

To just what extent can the results obtained in monkeys, particularly with the intraspinal test predict the safety of attenuated poliovirus for man? This all-important question is difficult, if not impossible, to answer at the present time Since monkeys now constitute the only available laboratory yardstick, no doubt the association of vaccine strains with the lowest possible degree of neurovirulence in these animals is desirable, so long as it does not entail undue loss of antigenicity But before a test system can be accepted for routine use, it must be reproducible within a reasonable range of deviation, for the formulation of realistic minimum requirements

TABLE 11 INTRACEREBRAL MONKEY VIRULENCE OF SUCCESSIVE LOTS OF TYPE 2 ORAL POLIO VACCINE, AT DIFFERENT DILUTIONS

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
200	7.5	0/9	0/10	1/10	2?/9	1/10	0/10	1+2?/9	1/10	0/10
213	7.5	1/8	0/9	0/9	6+2?/10	3+4?/9	7/10	4+3?/10	4+2?/9	3+4?/10
216	7.5	1/20	1+1?/19	1+1?/18	3+9?/20	5+3?/20	3+5?/19	7+5?/20	6+5?/20	4+4?/19
217A	6.8	2/19	0/8	0/8	5+5?/20	6+2?/10	1?/10	7+3?/20	5+3?/10	2?/10
222	7.3	0/10	0/5	0/6	2?/10	1?/6	2?/6	2?/10	2?/6	3?/6
227	7.3	0/9	0/6	0/6	9+1?/10	6/6	5/6	9/9	6/6	5+1?/6
228	7.0	0/10	1/6	0/6	9/9	6/6	4/6	9/9	5/6	4/6
240†	7.3	0/8	0/5	0/4	1/10	0/6	0/6	1/10	0/6	0/5
241†	7.6	0/5	1/5	0/5	1?/10	1+1?/6	2/6	1?/10	2/6	2/6
242†	7.4	0/7	0/5	0/5	1?/10	1+1?/6	1/6	3?/10	1/6	2/6
243†	7.4	0/8	0/6	0/5	1/10	0/6	0/6	1/10	0/6	1/6
Totals		4/113	3+1?/84	2+1?/82	34+23?/128	29+12?/91	22+8?/91	39+19?/127	30+12?/91	21+14?/90
Percent		3.5	4.7	3.6	44.5	45.0	33.0	45.7	46.1	39.0
Percent of Total		3.9			41.3			43.6		

* Log TCD_{50} per ml.
† Scored histopathologically by method B All others scored by method A

Table 10. Intraspinal Monkey Virulence of Successive Lots of Type 1 Oral Polio Vaccine, at Different Dilutions

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
110	7.7	6/9	4+1?/8	3+1?/10	7+1?/10	3+1?/10	3+3?/10	8+1?/10	5/10	6+2?/10
114	7.5	3/10	4/10	4/10	2?/9	6+2?/8	4+2?/10	4/9	9/10	7/10
115	7.3	3/8	3/8	0/8	3/8	2+1?/8	0/8	5/8	4/8	0/8
120	7.7	3/8	4/8	0/7	3+2?/8	3+1?/8	2/8	6/8	4/7	4/8
121	8.2	2/8	3/6	0/7	1+2?/8	2+1?/8	1?/8	3+2?/8	4+1?/8	1?/8
126	7.5	6/22	3/12	7/17	4+5?/22	3+1?/11	5+2?/18	14+1?/21	9/12	10+2?/18
140	7.7	11/29	10/15	9/18	6+2?/20	3+1?/11	5+2?/12	16+1?/20	9/12	9+1?/12
162P†	7.3	2+1?/10	3/6	0/6	2+1?/10	3/6	0/6	8/10	3+1?/6	1+1?/6
Totals		36+1?/104	34+1?/73	23+1?/83	26+15?/95	25+8?/70	19+10?/80	64+5?/94	47+2?/73	37+7/80
Percent		35.5	47.9	28.8	43.1	47.1	36.2	73.4	67.1	55.0
Percent of Total			37.0			42.0			65.5	

* Log TCD_{50} per ml.
† Scored histopathologically by method B. All others scored by method A.

TABLE 13 INTRASPINAL MONKEY VIRULENCE OF SUCCESSIVE LOTS OF TYPE 2 ORAL POLIO VACCINE, AT DIFFERENT DILUTIONS

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
200	7 5	2/8	1/8	0/8	6+1?/10	2+5?/10	2+2?/9	9/10	6+3?/10	3+1?/8
213	7 5	4/8	1/8	0/8	6/8	5+2?/8	1/8	6/7	7/8	2+1?/8
216	7 5	4/8	1/8	0/8	4+1?/6	3?/8	2?/8	5/6	2+1?/8	2/8
217A	6 8	6/16	7/16	4/14	9+3?/16	12+1?/16	4+2?/16	13/16	13/16	7/16
222	7 3	3/10	4/6	2/6	5+2?/10	1+2?/6	1+3?/6	9/10	4/6	6/6
227	7 3	5/9	4/6	3/6	5+2?/10	5+1?/6	3+2?/6	7/10	6/6	4+1?/6
228	7 0	4/10	4/5	3/5	9+1?/10	5+1?/6	2+4?/6	9+1?/10	6/6	4+2?/6
240†	7 3	4/10	1/4	1/3	5/10	3/6	1/6	7/10	3/6	3/6
241†	7 6	7/9	4/6	2/6	5+2?/10	4+1?/5	3+2?/6	10/10	5/5	5/6
242†	7 4	7/9	4/5	2/5	8/10	3/6	4/6	9/10	6/6	6/6
243†	7 4	3/10	0/4	0/6	6/9	1/5	1/6	6/9	2/5	2/6
Totals		49/107	31/76	17/75	62+12?/109	41+16?/82	22+17?/83	90+1?/108	60+4?/82	44+5?/82
Percent		45 0	41 0	23 0	73 4	69 5	47 0	84 3	78 0	59 7
Percent of Total			37 6			64 2			75 0	

* Log TCD_{50} per ml
† Scored histopathologically by method B All others scored by method A

TABLE 12. INTRACEREBRAL MONKEY TEST. POLIOMYELITIS HISTOPATHOLOGICAL SCORE OF REPRESENTATIVE LOTS OF TYPE 2 VACCINE

Lot No	Dilution Inoculated	No of Monkeys Examined	Polio Histopathol Score (1)—Number of Monkeys with													
			Cervical Lesions							Lumbar Lesions						
			0	±	1+	2+	3+	4+	5+	0	±	1+	2+	3+	4+	5+
213*	Undil	10	2	2	3	1	2			3	2	1	4			
	10^{-1}	9	2	4	3					3	2	3	1			
	10^{-2}	10	3		3	4				3	4	1	2			
216*	Undil	10	5	3	2					5	1	4				
	10^{-1}	10	5	2	2	1				5	1	3	1			
	10^{-2}	9	4	3	2					4	3	2				
217A*	Undil	20	10	5	3	2				10	3	4	3			
	10^{-1}	10	2	2	5	1				2	3	4	1			
	10^{-2}	10	9	1						8	2					
227*	Undil	10		2	3	5					2	2	4	2		
	10^{-1}	6			5	1						2	4			
	10^{-2}	6	1		3	1	1				2		2	2		
213†	Undil	10	9			1				9				1		
	10^{-1}	6	6							6						
	10^{-2}	6	6							5		1				
	Totals	142	64	24	34	17	3			63	25	27	22	5		
	Percent		45.1	16.9	23.9	12.0	2.1			44.4	17.6	19.0	15.5	3.5		

(1)
0=No cellular loss
±=Less than 10% loss
1+=10-20% loss
2+=20-40% loss
3+=40-60% loss
4+=60-80% loss
5+=80-100% loss

* Scored histopathologically by method A
† Scored histopathologically by method B

Table 15 Intraspinal Monkey Virulence of Successive Lots of Type 3 Oral Polio Vaccine, at Different Dilutions

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
310†	7 5	0/5	2?/5	3/5	1/5	1+3?/5	1/5	1+1?/5	5/5	2+1?/5
318†	7 7	3/8	3/8	0/8	3/8	3+1?/8	1/8	7/8	5/8	5/8
319†	7 5	1/8	1/8	1/8	2?/8	3?/8	1/8	4+1?/8	1+1?/8	2+1?/8
334	6 8	4/9	3/5	1/6	0/10	2/6	0/6	7/10	5/6	2/6
341	8 0	1/10	2/6	3/6	1+2?/10	2?/6	1?/6	5+1?/10	4/6	3+2?/6
342	7 6	5/10	2+1?/5	4/6	3+3?/9	5+1?/6	4+2?/6	9+1?/10	6/6	5+1?/6
344	7 7	1/10	0/5	0/5	1/10	4/6	0/6	5+2?/10	5+1?/6	1+1?/6
352P	7 2	0/9	4/6	4/6	1/10	3+1?/6	2+1?/6	8+1?/10	6/6	6/6
360P	7 1	0/10	0/4	0/6	2/9	1?/6	1/6	8/9	6/6	4/6
361P	7 4	4/9	2/6	3/6	5+2?/9	3/6	2/6	8/9	4/6	5/6
362P	7 6	7/9	2+1?/6	1+1?/5	4/10	0/6	1/6	10/10	4/6	4/6
Totals		26/97	19+4?/64	20+1?/67	21+9?/98	21+12?/69	13+4?/69	72+7?/99	51+2?/69	39+6?/69
Percent		26 7	36 0	31 3	30 5	47 8	24 6	79 7	70 8	65 2
Percent of Total		30 7			34 9			74 6		

* Log TCD_{50} per ml
† Scored histopathologically by method A. All others scored by method B

TABLE 14. INTRACEREBRAL MONKEY VIRULENCE OF SUCCESSIVE LOTS OF TYPE 3 ORAL POLIO VACCINE, AT DIFFERENT DILUTIONS

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio†					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
310‡	7 5	0/5	0/4	—	0/5	0/5	—	0/5	0/5	—
318‡	7 7	0/8	0/9	0/9	0/10	0/10	0/9	0/10	1?/10	0/9
319‡	7 5	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
334	6 8	0/10	0/6	0/6	0/10	0/6	0/6	0/10	0/6	0/6
341	8 0	1?/10	0/6	0/6	1/10	0/6	0/6	1/10	0/6	0/6
342	7 6	0/9	0/6	0/5	0/10	0/6	0/6	0/10	0/6	0/6
344	7 7	0/10	0/6	0/5	0/10	0/6	0/6	0/10	0/6	0/6
352P	7 2	1?/10	0/6	0/6	6/9	1/6	1+2?/6	5+2?/10	1+3?/6	1+3?/6
360P	7 1	0/8	0/5	0/5	2/10	0/6	0/6	2+1?/10	1+1?/6	0/6
361P	7 4	0/10	0/5	0/6	2+2?/10	0/6	0/6	2/10	0/6	0/6
362P	7 6	1?/9	0/4	0/6	2?/10	1+1?/6	0/5	2?/10	0/6	1+1?/6
Totals		3?/99	0/67	0/64	11+4?/104	2+1?/73	1+2?/66	10+5?/105	2+4?/73	2+4?/67
Percent		3	0	0	14 4	4 1	4 5	14 3	8 2	8 0
Percent of Totals		1 2			8 6			11 0		

* Log TCD_{50} per ml
† Most lesions were scored by method B and consisted mainly of inflammatory reactions with little or no neuronal damage or loss
‡ Scored histopathologically by method A. All others scored by type B

TABLE 15 INTRASPINAL MONKEY VIRULENCE OF SUCCESSIVE LOTS OF TYPE 3 ORAL POLIO VACCINE, AT DIFFERENT DILUTIONS

Lot No	Titer*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}	Undil	10^{-1}	10^{-2}
310†	7 5	0/5	2?/5	3/5	1/5	1+3?/5	1/5	1+1?/5	5/5	2+1?/5
318†	7 7	3/8	3/8	0/8	3/8	3+1?/8	1/8	7/8	5/8	5/8
319†	7 5	1/8	1/8	1/8	2?/8	3?/8	1/8	4+1?/8	1+1?/8	2+1?/8
334	6 8	4/9	3/5	1/6	0/10	2/6	0/6	7/10	5/6	2/6
341	8 0	1/10	2/6	3/6	1+2?/10	2?/6	1?/6	5+1?/10	4/6	3+2?/6
342	7 6	5/10	2+1?/5	4/6	3+3?/9	5+1?/6	4+2?/6	9+1?/10	6/6	5+1?/6
344	7 7	1/10	0/5	0/5	1/10	4/6	0/6	5+2?/10	5+1?/6	1+1?/6
352P	7 2	0/9	4/6	4/6	1/10	3+1?/6	2+1?/6	8+1?/10	6/6	6/6
360P	7 1	0/10	0/4	0/6	2/9	1?/6	1/6	8/9	6/6	4/6
361P	7 4	4/9	2/6	3/6	5+2?/9	3/6	2/6	8/9	4/6	5/6
362P	7 6	7/9	2+1?/6	1+1?/5	4/10	0/6	1/6	10/10	4/6	4/6
Totals		26/97	19+4?/64	20+1?/67	21+9?/98	21+12?/69	13+4?/69	72+7?/99	51+2?/69	39+6?/69
Percent		26 7	36 0	31 3	30 5	47 8	21 6	79 7	76 8	65 2
Percent of Total			30 7			33 9			74 6	

* Log TCD_{50} per ml
† Scored histopathologically by method A. All others scored by method B

Of the two modes of monkey inoculation, the intracerebral test best meets this specification It is both reproducible and consistent, and should provide a well-founded basis for acceptable criteria of residual neurovirulence. Surely a virus which fails to paralyze and causes only minimal histopathological changes when inoculated into monkeys intracerebrally in amounts equalling or exceeding 10 million TCD_{50} can be regarded as attenuated several million-fold, in view of the fact that a virulent Type 1 field strain inoculated by the same route did paralyze monkeys with as few as 2 TCD_{50}[8]

Admittedly, some of the early Type 2 vaccine lots were more active than is desirable when inoculated into monkeys intracerebrally And although their safety in the field remains unquestioned, steps have been taken to reduce the activity of later lots to a minimum or even virtually to eliminate it With the more recent batches of Type 2 vaccine this has been accomplished, to a great extent

Compared with the intracerebral test, results with the intraspinal technique have been much less consistent In fact, considerable variation has been reported from different laboratories[7] In an attempt to explain these differences, the following factors should be taken into account

1 The method of scoring lesions In our experience, Methods A and B previously described give easily comparable results so far as the intracerebral test is concerned, but not with the intraspinal test Placement of the inoculum in the anterior horn was not considered crucial when Method A was employed That method has been used extensively in our laboratory, and gave scores consistently lower than did Method B, which was adopted only recently in order to obtain results comparable to those of other workers.

2. The size of the needle used for inoculation Scores obtained with a smaller (27-gauge) needle were consistently higher than those with a 20-gauge needle, probably because the former expels the virus preparation at greater speed

3. The number of inoculations More extensive lesions were obtained with the "multiple jab" method, as employed by Melnick, than with the single-inoculation technique of Sabin, a fact already stressed by Melnick[7] Obviously, with the former method the chances of "hitting" the anterior horn, often repeatedly, are greater than with the latter.

4 The placement of the inoculum within the spinal cord In his description of the intraspinal test, Sabin emphasized the necessity of inoculating the virus within the anterior horn, in direct contact with the large motor nerve cells, as would be indicated by a needle track extending from the posterior or posterior lateral aspect of the cord through the anterior horn to the anterior lateral cord surface[7] However, histological determination of whether the inoculum was being placed in the anterior horn or in the posterior position of the track often proved difficult, and a difference in position of a few micra would result in considerable difference in the scoring of the lesion.

It may be pertinent at this point to raise the question of the significance of a track outside the anterior horn Figure 1 shows, side by side, sections from the lumbar cords of two monkeys inoculated with the same dilution of Type 2 vaccine In the section to the right the needle track is properly placed in the gray matter, the adjacent area shows marked inflammation, and in both horns almost all neurons are destroyed In the one to the left the track is clearly visible in the white matter, and in the adjacent anterior horns all neurons have been spared Sabin proposed, in his discussion of the intraspinal test, that in the final evaluation of results those animals yielding cord sections with the needle track in the white matter be disregarded[7] But instead, shouldn't a finding such as that depicted in the photograph raise the following question How virulent or invasive can a poliovirus be if it cannot bridge a gap of a few millimeters, when virulent poliovirus placed anywhere in the central nervous system, and even extraneurally, is well known to be capable of crossing more formidable barriers in seeking out and destroying susceptible neurons?

5 The histological interpretation of circumscribed lesions as specifically due to virus action Typical nonspecific traumatic lesions are shown in Figure 2 The lesion on the left is undoubtedly traumatic, as indicated by the extensive formation of scavenger cells and the secondary inflammatory reaction at the periphery of the softening On the right, a similar lesion can be seen in the anterior horn which might be

interpreted either as purely traumatic or as a mixture of trauma and specific viral effect

In other instances, non-specific reactions to foreign material may present similar difficulty in interpretation. The occurrence of such reactions following intraspinal inoculation is well established In Figure 3, for example, the presence of large multinucleated cells indicates that the inflammatory reaction was caused by foreign bodies and not by poliovirus In other cases, however, the interpretation might remain in doubt

6. Finally, the possibility of the introduction with the inoculum of a bacterial agent of low virulence. The formation of small abscesses with an accumulation of polymorphonuclear cells was seen occasionally, as were perivascular infiltrations which included large numbers of leucocytes These hematogenous elements are well known to appear only in the very first phase of the poliomyelitis process and to disappear almost completely by the third week (the time at which the monkeys were sacrificed)

At this point it may be of interest to report *results of recent experiments intended to evaluate* the reproducibility of the intraspinal test when using an inoculation technique as uniform as possible This was done in two ways by repeated testing of the same vaccine lot, or by simultaneous testing of vaccine lots prepared at the same time *from the same seed* lot Results of these tests are summarized in Tables 16 and 17

In Table 16, three parallel lots of Type 3 vaccine, inoculated by means of a 20-gauge needle, gave paralytic ratios of 6/22 monkeys for the first, 12/21 for the second and 1/20 for the third This variation is notable even if as proposed by Sabin, results exclude those animals which, on histological examination showed no needle track or showed tracks in *the* white matter. The respective rates for the three lots then become 6/16, 12/22 and 1/17 or percentages of 37.5, 54.5, and 6, a noteworthy variation The discrepancy may reflect the inherent irreproducibility of the test despite uniform technique, or may indicate that neurovirulence of the virus in different production lots can fluctuate appreciably, despite uniform production conditions Should the latter be the case it becomes imperative to test several serial lots of any new vaccine before its intraspinal performance can be properly evaluated

However, the importance of taking into account the first possibility mentioned above—irreproducibility of the test—is suggested by the experiment described in Table 17 Here, the same lot of Type 3 virus was tested intraspinally, twice with a 20-gauge and once with a 27-gauge needle Excluding the animals in which the inoculum was not placed in the gray matter, paralytic ratios in the two 20-gauge needle tests were 1/17 (6%) and 8/14 (57%), respectively almost a *10 fold difference The* 27-gauge-needle test confirmed the greater reaction elicited by this technique, almost 100 per cent of the animals showing some degree of limb weakness or paralysis

While the intraspinal technique undoubtedly *can be a valuable experimental tool for the selec*tion of poliovirus variants completely devoid of their affinity for neurons, the findings reported above give reason for believing that it should not be a requirement for oral poliomyelitis vaccine First the true relation of the results of the *test to the vaccine's safety for man is obscure* Second, how could the safety of a vaccine be measured with any degree of uniformity by a test subject to such wide variations which, in all probability, would be carried out by many hands in several laboratories?

This laboratory recently has segregated virus strains of notably less intraspinal activity derived from isolates other than those described in this report However, in view of the question able significance of the intraspinal test it would be unwise to consider at this time the substitution of untried candidates for strains already proved safe in the field Furthermore in the final assessment of vaccine strains, factors other than monkey neurovirulence must also be considered Antigenicity for one is as important as safety, and the strains reported on here have been shown to elicit very satisfactory immune *responses while viruses with less* intraspinal activity might prove immunogenically inferior In addition, there is increasing evidence that the Lederle strains can be administered simultaneously in a trivalent preparation, with immunogenic results equivalent to those following monovalent feedings*

And isn't it in man himself that the safety of oral poliomyelitis vaccine is ultimately estab-

Table 16. Variation of Intraspinal Monkey Virulence on Simultaneous Testing of Consecutive Lots of Type 3 Oral Polio Vaccine

Lot No	Needle Gauge	Dilution	Paralytic Ratio	Polio Histopathological Ratio with Needle Track.					
				Absent		in White Matter		in Gray Matter	
				C	L	C	L	C	L
311	20	Undil	1/10	0/3	0/3	—	—	1+2?/7	5+1?/7
		10^{-1}	2/6	0/2	0/2	—	—	2?/4	4/4
		10^{-2}	3/6	0/1	0/1	—	—	1?/5	3+2?/5
312	20	Undil	5/10	—	—	—	—	3+3?/9	9+1?/10
		10^{-1}	2+1?/5	—	—	—	—	5+1?/6	6/6
		10^{-2}	4/6	—	—	—	—	4+2?/6	5+1?/6
314	20	Undil	1/10	0/1	0/1	0/1	0/1	1/8	5+2?/8
		10^{-1}	0/5	—	—	—	—	4/6	5+1?/6
		10^{-2}	0/5	0/2	0/2	0/1	0/1	0/3	1+1?/3

Table 17. Variation of Intraspinal Monkey Virulence on Repeated Testing of Same Lot of Type 3 Oral Polio Vaccine (Lot 7-1233-344)

Test No	Needle Gauge	Dilution	Paralytic Ratio	Polio Histopathological Ratio with Needle Track: Absent		In White Matter		In Gray Matter	
				C	L	C	L	C	L
1	20	Undil	1/10	0/1	0/1	0/1	0/1	1/8	5+2?/8
		10^{-1}	0/5	—	—	—	—	4/6	5+1?/6
		10^{-2}	0/5	0/2	0/2	0/1	0/1	0/3	1+1?/3
2	20	Undil	4/10	0/2	0/2	0/1	0/1	4/7	7/7
		10^{-1}	3/6	0/1	0/1	—	—	4/5	5/5
		10^{-2}	1/4	0/4	0/4	—	—	1/2	2/2
3	27	Undil	9/10	—	—	1/1	1/1	7/9	7/9
		10^{-1}	5+1?/6	0/1	0/1	0/1	0/1	0/4	4/4
		10^{-2}	1+2?/5	—	—	0/2	2/2	1/4	3/4

lished, however attenuated a poliovirus may be found to be in the laboratory? Data accumulated so far with the Lederle strains of virus are beginning to answer this question Complete results of all clinical trials carried out with these strains are not yet available, but those now at hand are most reassuring.

Of approximately 500,000 persons fed Type 1 virus (Table 18), antibody surveys in four trials comprising 65,429 individuals, or about 12 per cent of the total fed, revealed that 13,959 of them, or 21 3 per cent, lacked Type 1 antibodies The rate of homotypic negatives varied widely among areas, ranging from 10 per cent of the study group in Cuba to 44 per cent in Minnesota Assuming the applicability of the lowest rate, 10 per cent, to all persons fed, it can be estimated that of the total number of 500,000, 50,000 were negative for Type 1 at the time of ingestion of the vaccine. In addition, there were in the four completed trials, an estimated 2,750 triply negative individuals These rates also differed substantially, ranging from 1 per cent in Cuba, or 3 per cent in Nicaragua, to 13 per cent in Andes, Colombia, and 20 5 per cent in the Minneapolis study.

Although the rate of triple negatives before ingestion remains the same for Type 2 vaccine, the rate of homotypic negatives in the four trials was lower 7 208 persons, or 11 per cent of the total (Table 19) Again, rates differed greatly according to area, ranging from 5 5 per cent in Cuba to 33 per cent in Andes, Colombia

Table 20 summarizes the results obtained with Type 3 virus The number of homotypic negatives was estimated at 14,072 representing a rate very close to that for Type 1

The numbers of homotypic and triple negatives in these studies appear to be sufficiently great to have caused some difficulties had the viruses used not been attenuated However, as will be reported by others, not a single proved case of paralysis attributable to the vaccine was reported from any of the trial areas This was equally true for Type 2 virus, despite its somewhat greater activity in monkeys It should also be borne in mind that the numbers cited apply only to persons vaccinated, without taking into account the probably even greater numbers of the satellite infections that undoubtedly have occurred as a result of contact exposure

Summary and Conclusions

Brief histories of the three type strains of Lederle attenuated polioviruses have been presented, and production and testing data pertaining to 31 lots of experimental oral vaccine have been described In all, 113 liters of Type 1 vaccine, 120 of Type 2, and 143 of Type 3 were prepared and completely tested, yielding quantities sufficient for 4½, 2½ and 4½ million human immunizations, respectively.

Only an occasional bacterial contaminant, and no CNS, herpes, simian, measles or B virus was encountered. The presence of TB was also excluded from all vaccine lots, both by guinea pig inoculation and in culture media.

Intracerebral monkey inoculations of these vaccines produced over all paralytic rates of 1 5 per cent for Type 1, 3 9 per cent for Type 2, and 1 2 per cent for Type 3 Histopathological changes following inoculation by this route were virtually absent with Type 1 and Type 3 vaccine lots Although early batches of Type 2 were associated with some neuronal loss, more recent lots have produced almost none

Despite the somewhat greater activity of some Type 2 vaccines when inoculated intracerebrally, in monkeys inoculated intraspinally activity of lots of all three types was comparable, producing similar over-all paralytic rates Paralysis induced by this route of inoculation was usually non-progressive and limited to one or both lower limbs, and only an occasional animal died within the 21-day observation period Histopathological lesions were observed in most intraspinally inoculated animals whether or not they were paralyzed

Each of the three Lederle strains of virus has been fed to approximately 500,000 persons in several trials in the U S A and Central and South America In completed trials comprising 65,429 individuals, or 12 per cent of the total fed, estimated homotypic negatives amounted to 13 959 or 21 3 per cent for Type 1, 7,208 or 11 per cent for Type 2, and 14 072 or 22 per cent for Type 3. In addition there were, among the 65,429 persons surveyed serologically, an estimated 2 750 triple negatives In not a single instance was a proved case of paralysis or disease attributed to the vaccine.

The intraspinal test has been discussed with regard to its reproducibility and its significance

Table 18. Field Experience with Successive Lots of Type 1 Oral Polio Vaccine

Lot No	Log. TCD_{50} Fed	Country or State	No of Persons		Estimated Negatives			
					Homotypic		Triple	
			Fed	Tested	No	%	No	%
110	5 0	Minnesota 2nd Trial	551	551	241	41 0	113	20 5
110	5 0	Uruguay	45 000					
115	5 7	Andes, Colombia	7,378	452	2,088	28 3	952	12 9
115	5 7	Costa Rica	28,000					
115	5 7	Uruguay	9 000					
120	5 1	Cuba	2,000	274	200	10 0	20	1 0
120	5 1	Uruguay	130,000					
121	5 5	Medellin, Colombia	133 000					
121	5 5	Haiti	5,000					
121	5 5	Nicaragua	55 500	505	11 430	20 6	1 665	3 0
121	5 5	Uruguay	60 000					
		Total Fed	475,429					
		Total Surveyed	65 429	1,782	13 959	21 3	2,750	4 2

Table 19 Field Experience with Successive Lots of Type 2 Oral Polio Vaccine

Lot No	Log TCD_{50} Fed	Country or State	No of Persons		Estimated Negatives			
					Homotypic		Triple	
			Fed	Tested	No	%*	No	%*
213	5 2	Andes, Colombia	7,122	452	2,357	33 1	952	12 9
213	5 2	Minnesota 2nd Trial	551	551	141	25 5	113	20 5
213	5 2	Uruguay	160,000					
216	5 5	Medellin, Colombia	48 000					
216	5 5	Nicaragua	55,500	505	4,600	8 3	1,665	3 0
216	5 5	Uruguay	90,000					
217A	5 4	Medellin, Colombia	85,000					
227	5 7	Cuba	2,000	274	110	5 5	20	1 0
227	5 7	Costa Rica	75,000					
227	5 7	Haiti	5,000					
		Total Fed	528,173					
		Total Surveyed	65,173	1,782	7,208	11 0	2,750	4 2

* Percentages are based on total number of persons starting course of feedings

TABLE 20. FIELD EXPERIENCE WITH SUCCESSIVE LOTS OF TYPE 3 ORAL POLIO VACCINE

Lot No	Log TCD_{50} Fed	Country or State	No of Persons		Estimated Negatives			
					Homotypic		Triple	
			Fed	Tested	No	%*	No	%*
318	5 3	Andes, Colombia	6,977	457	2,728	39 1	952	12 9
318	5 3	Cuba	2,000	274	356	17 8	20	1 0
318	5 3	Medellin, Colombia	50,000					
318	5 3	Minnesota; 2nd Trial	551	551	318	57 7	113	20 5
318	5 3	Nicaragua	55,500	505	10,670	19 4	1,665	3 0
318	5 3	Uruguay	58,000					
319	5 5	Medellin, Colombia	83,000					
319	5 5	Haiti	5,000					
319	5 5	Uruguay	192,000					
		Total Fed	453,028					
		Total Surveyed	65 028	1 787	14,072	21 6	2,750	4 2

* Percentages are based on total number of persons starting course of feedings

FIG 1 INTRASPINAL INOCULATION OF CYNOMOLGUS MONKEYS
Left Needle track in white matter—neurons intact—no inflammation
Right Needle track in gray matter—neurons destroyed—marked inflammation

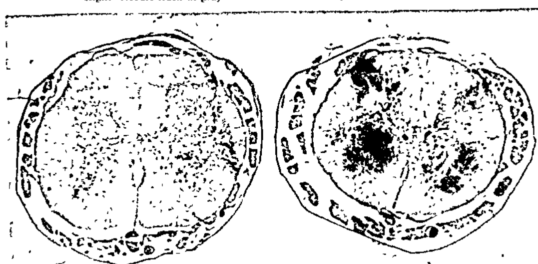


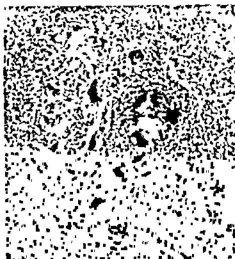
FIG. 2 INTRASPINAL INOCULATION OF CYNOMOLGUS MONKEYS

Left Traumatic lesion with formation of a small area of softening and intact neurons in anterior horns
Right Extensive area of softening in gray matter with secondary inflammation and possible specific viral effect



FIG. 3 INTRASPINAL INOCULATION OF CYNOMOLGUS MONKEYS

Section of lumbar enlargement showing large multinucleated cells in the gray matter indicating inflammatory reaction due to foreign bodies and not to poliovirus



in relation to safety of the vaccine virus for man. The more consistent and more readily carried out intracerebral monkey inoculation has been proposed as the basis for criteria of residual monkey virulence to be used for release of oral vaccine

Efforts to improve Lederle vaccine strains in every possible way are continuing. Meanwhile the utility of the present strains, in view of the obvious need for a better solution to the problem of immunization against paralytic poliomyelitis, has been demonstrated by the mounting evidence of their effectiveness and safety and the absence of significant undesirable reactions

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5. THE ROLE OF MARKERS OF POLIOVIRUS IN ATTEMPTS TO IDENTIFY STRAINS ISOLATED FROM MAN DURING A MASS VACCINATION PROGRAM

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DR KOPROWSKI Research on genetic markers of the poliomyelitis virus has been stimulated considerably by the interest in linking some of these markers with decreased virulence of the virus. The studies of genetic markers, although *practical in intent, have resulted in the accumulation of data which are of fundamental significance in the field of virus genetics*

Table 1 lists 7 different markers of polio virus Of particular significance to this discussion are those of Dulbecco and Vogt,[1] Kanda and Melnick,[2] McBride[3] and Lwoff[4] Dulbecco and Vogt[1] observed that when certain strains of polio virus are seeded on monkey kidney *monolayers under an agar overlay maintained in medium of low bicarbonate content, there is a delay in the appearance of plaques* In an alkaline medium all strains of polio virus tested show more or less equal plating efficiency In *an acid medium, however, strains encountered in nature may show similar plating efficiency,* but those mutants which have a decreased pathogenicity for Rhesus monkeys grow at a markedly slower rate than they do in an alkaline medium.

TABLE 1. MARKERS OF POLIOMYELITIS VIRUS WHICH CAN BE USED IN THE STUDY OF MUTANTS

1 PLATING EFFICIENCY UNDER AGAR OF LOW BICARBONATE CONTENT
2 COMPARATIVE GROWTH CHARACTERISTICS ON MK AND MS CELLS
3 SEROLOGICAL SPECIFICITY (COMPARATIVE VELOCITY OF NEUTRALIZATION BY SERA)
4 THERMOSENSITIVITY
5 PLAQUE SIZE ON MONOLAYER OF MONKEY KIDNEY
6 RELATION TO PRESENCE OF CYSTINE IN TISSUE CULTURE MEDIUM
7 INHIBITION BY NORMAL BOVINE SERUM

A study by Kanda and Melnick[2] indicates that *certain strains of polio virus grow at different* rates when cultivated on primary monkey kidney monolayer and on the MS cell line (a stable tissue culture line originally derived from the *renal epithelium of a monkey*) Strains pathogenic for monkeys injected intracerebrally grow equally well on the MS cell line and monkey kidney Certain strains with low pathogenicity for monkeys give lower titers in MS tissue culture than in monkey kidney

McBride's kinetic studies[3] *on the serum neutralization* of polio virus show that each strain can be clearly identified by its homologous immune serum In McBride's neutralization test, methods developed for the study of bacteriophages have been applied to polio virus: A hyper*immune serum is mixed with a given number of* PFU/ml of the virus and incubated at 37°C At several intervals between 0 and 15 minutes of incubation an aliquot of the mixture is removed and the number of PFU present determined Applying a formula developed for the rates of neutralization reactions, a K value is determined. *Comparison of K values enables McBride to determine the serologic specificity of* polio virus strains within a given type He has also been able to show that the *antigenic character of a given virus is highly stable*

Although host resistance and heat susceptibility of various polio virus strains have been studied in the past, only recently has it been *shown, by Lwoff, that various strains of polio* virus have relatively specific thermosensitivity A highly neurovirulent strain of poliomyelitis which Lwoff[4] studied is relatively insensitive *to high temperature and also grows in tissue culture at a relatively rapid rate* when kept at a high temperature In contrast, strains with a

low neurovirulence are extremely sensitive to high temperature. This marker, again, is probably associated with susceptibility to high and low pH.

I will not discuss the remaining three markers because they are not directly applicable to the study I would now like to present.

The two attenuated strains used in the present study are the CHAT strain of Type 1 virus and the Fox 3 strain of Type 3. The origin of these strains has been adequately described in the literature,[7] and to save time I shall concentrate on the presentation of their markers. Table 2 summarizes the distinguishing characters of the CHAT and Fox 3 strains in respect to four of the poliovirus markers. Both strains have low plating efficiency under low bicarbonate content and acid pH. This property is referred to as a *d* character. These strains have much lower titers when grown on MS cells than on monkey kidney cells. As indicated by the K value, there is a specific velocity of the neutralization reaction when a hyperimmune serum against either CHAT or Fox 3 strains is mixed with the virus and the kinetics of the neutralization test reaction determined. Finally, the CHAT strain, according to Lwoff's[8] studies, is very sensitive to high temperatures.

TABLE 2. DISTINGUISHING CHARACTERS OF ATTENUATED STRAINS CHAT AND FOX 3

A. *LOW* PLATING EFFICIENCY UNDER LOW BICARBONATE CONTENT (*d*)

B. *LOW* TITERS ON MS CELLS AS COMPARED TO MK CELLS (MS)

C. *SPECIFIC* VELOCITY OF NEUTRALIZATION REACTION

D. *HIGHLY* SENSITIVE TO HIGH TEMPERATURE

Studies of markers are highly relevant to the subject of mass vaccination with live attenuated strains of poliomyelitis virus. It is important to *detect changes in the virus characteristics after* one or several passages through the human intestinal tract, and to identify any wild mutants if live strains are administered during an epidemic. One of the most often used arguments against the use of live virus as a method of oral immunization against poliomyelitis is that such strains might change rapidly after passage through the human intestinal tract and, according to the more irrational opponents of live virus vaccination, that they might even start an epidemic rather than prevent or halt one.

An opportunity for some "detective" work on markers of attenuated strains fed before, during and after an epidemic of poliomyelitis was presented to us last winter. My collaborators, Drs. Plotkin and Lebrun, will discuss the details of a field trial and the protection given by vaccination during an epidemic in the Belgian Congo. I will present data on the properties of polio strains in relation to that epidemic. Infants and children less than 5 years old were studied since they were the only ones to contract the disease during the epidemic.

Stools obtained 24 hours after a child showed signs of polio infection were processed either at The Wistar Institute or in the laboratory of Dr. Van de Putte in Leopoldville. A summary of these results is presented in Table 3. A stool specimen was obtained from each of 18 children: Type 1 virus was recovered in 14 cases and Type 2 in one. Three specimens failed to yield an agent cytopathogenic for monkey-kidney tissue culture. Three stools obtained from children previously vaccinated against poliomyelitis yielded Type 1 virus. I have no information as to how many stool specimens were tested by Dr. Van de Putte, but he has reported recovery of Type 1 virus from 16 specimens, Type 3 from one and Coxsackie from four.

It would be interesting at this point to present data obtained by Dr. Klaus Hummeler, of Children's Hospital in Philadelphia,[9] in which the results of the complement fixation test with heated polio antigen were correlated with the isolation of the virus from stool specimens (Table 4). Seven serum specimens drawn during an acute phase of the disease specifically fixed complement in the presence of Type 1 virus heated antigen. In four cases the diagnosis was confirmed by the isolation of Type 1 virus from stool specimens. The results of complement *fixation tests with the 5 remaining specimens* were rather equivocal, since antibodies were detected in the presence of Type 1 and Type 2, and in one case in the presence of Type 3 antigens. It was interesting to note, however, that in the single case with a serum titer higher against Type 2 antigen than against the two other types, Type 2 virus was obtained from the fecal specimens. In the remaining four cases the serum titer against Type 1 antigens was slightly higher

TABLE 3. ISOLATION OF POLIO VIRUS FROM FECES OF CLINICAL CASES IN LEOPOLDVILLE

Tissue Culture Type	Group of Subjects	Number Specimens Tested	Virus Isolation				
			None	1	2	3	Coxsackie
MK	Non-vac	18	3	14	1	0	0
	Vac	3	0	3	0	0	0
*HeLa**	Non-vac	*	*	*16*	*0*	*1*	*4*

* Results obtained by Van de Putte in Leopoldville

than against the other antigens. In all four cases Type 1 virus was isolated The results of this study seem to indicate that the presence of antibodies against Type 1 virus heated antigen is of great diagnostic value If antibodies against one or two other types of poliomyelitis *antigens are present, the results are somewhat* less significant

There were three vaccinated children with disease diagnosed as poliomyelitis Two of them had been vaccinated months before the epidemic started However one child had received the

TABLE 4 RESULTS OF COMPLEMENT FIXATION TEST ON SERA OBTAINED DURING AN ACUTE PHASE OF ILLNESS FROM CHILDREN IN THE *LEOPOLDVILLE AREA*

Child	Complement Fixation Test* Reciprocal of Serum Titer Against Polio Type Antigen			Isolation of Fecal Virus (Type)
	1	2	3	
Di	>128	<8	<8	*I*
Be	32	<8	<8	I
Bav	16	<8	<8	I
Vu	8	<8	<8	None
Li	64	<8	<8	I
Lu	64	<8	<8	None
Ts	>128	>128	<8	I
Ma	128	64	<8	I
Mi	64	128	64	II
Ka	64	32	8	I
Tay	32	16	<8	I

* Performed by Dr Klaus Hummeler

CHAT strain six days before the onset of fever It was obviously of great interest to identify the virus excreted by this child The strains chosen for study included that of the child just mentioned, two other Belgian Congo strains, one recovered during an epidemic at the same time *from a non-vaccinated child and one from a* child vaccinated several months previously, two vaccine lots of CHAT virus, and specimens known as first and third passage of CHAT virus in the human intestinal tract which had been obtained under well controlled, non-epidemic, conditions

All specimens were passaged once in monkey kidney-tissue culture. The preparations were titrated comparatively in tubes containing either the primary monkey kidney culture or the MS established stable line The results are shown in Table 5 The tissue culture titers of the CHAT vaccine lots and of CHAT virus after one or three passages through the human intestinal tract are two logs higher in monkey-kidney tissue culture than in the MS culture. In marked contrast, the wild strains isolated from the non-vaccinated child in Leopoldville and from the *two vaccinated children, including* the one vaccinated six days before onset of fever grew equally well in the primary monkey kidney and in the MS cells Although the number of strains studied in this test was not statistically large, the results were so strikingly definite that we consider them significant

Even more convincing results were obtained from studies on the kinetics of neutralization tests, made in collaboration with Dr A Bernstein[*] Figure 1 presents data on such studies for the CHAT hyperimmune rabbit serum against CHAT and Mahoney viruses respectively Ob-

6. CHARACTERISTICS OF LIVE POLIOVIRUS VACCINE PRODUCED IN THE INSTITUTE FOR POLIOMYELITIS RESEARCH, ACADEMY OF MEDICAL SCIENCES OF THE USSR, AND COMPARISON TO SABIN'S ORIGINAL VACCINE FROM ATTENUATED POLIOVIRUS STRAINS

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Dr. Chumakov (*presenting the paper*) 1 In the Institute for Poliomyelitis Research several lots of live poliovirus vaccine were prepared in December 1958-January 1959 from Professor Albert Sabin's attenuated strains The total volume of the vaccine is enough for immunization of 10 million people (over 100 liters of each of the three types)

Strains Used for Propagation

	Titer on 21 Nov, 1958 $TCID_{50}$ per 1 ml (cytopathogenic test)
Type 1—Lsc, 2ab	$10^{7.33}$
Type 2—P712, ch, 2ab	$10^{7.36}$
Type 3—Leon, 12a,b	$10^{7.2}$

These strains, which are the progeny of specially selected single plaques of the virus triply purified by successive plaque passages in monkey kidney cells under agar, were taken for propagation directly from vaccine vials (sent by Prof. A. Sabin in September 1958) or from the first passage of *these strains in monkey kidney* cell cultures

2. Method of vaccine production is analogous to that described by Sabin in the specification for his vaccine of 10 March 1958 Vaccine poliovirus strains grew in kidney cell cultures with the medium consisting of 0.5% lactalbumin hydrolysate and 2% heated calf serum in Earle's solution gassed with CO_2 to pH 7.5 About 10% of total normal (uninfected) kidney cell cultures were left as controls for the absence of spontaneous *cytopathogenic* viruses The 28-day observation of these cultures did not show any *spontaneous infection of monkey kidneys with* cytopathogenic viruses Bacteriologic sterility test of vaccine viruses before filtration was made in glucosated meat-peptone broth, Kitt Tarozzi medium and Sabouraud medium (at 37°C and 22°C)

Harvested virus-containing culture fluids were passed through plate separator ASG—3, followed by Seitz sterilizing filter pads "S" (pore diameter=0 5μ), and then distributed in 3 ml volumes in ampules, frozen at —20° —22°C and stored until use Three ml of vaccine contain 300 doses at 10^5 $TCID_{50}$ and over.

3 Vaccine virus titers after filtration were assayed by cytopathogenic test in roller kidney cell cultures (10 tubes per 0 5 log 10-fold dilution) on medium with 0 5% lactalbumin hydro*lysate, 2% calf serum in Earle's solution, pH 7 5* The following titers were established for the vaccine viruses

Type 1—$10^{7.6}$ $TCID_{50}$ in 1 ml
Type 2—$10^{7.83}$ $TCID_{50}$ in 1 ml
Type 3—$10^{7.22}$ $TCID_{50}$ in 1 ml

4 Type specificity of the produced lots of vaccine from Sabin's strains was checked in neutralization tests with standard rabbit antisera (antisera in 1 : 10 and 1 : 50 dilutions and viruses in 10^{-5} dilution). The results of the neutralization tests showed that all lots of live poliovirus vaccine prepared at the Institute were strictly type specific

5 Control for the absence in the final lots of live virus vaccine of non-poliomyelitis cytopathogenic agents was carried out by neutralization tests with undiluted virus mixed with undiluted

potent rabbit sera (with titer > 1 · 6000). In no case was any non poliomyelitis cytopathogenic agent found in the prepared lots of the vaccine

6. Control for the absence in the vaccine of lymphocytic choriomeningitis virus was done in white mice. Control for the absence in the vaccine of Coxsackie group of viruses was carried out by inoculation of suckling white mice. Absence in the vaccine of monkey B virus was tested by intracutaneous and subcutaneous inoculations of rabbits (6 rabbits per vaccine lot), and also by inoculation of monolayer rabbit kidney cell cultures Control for the absence of tubercle bacilli was done in guinea pigs (3 animals per lot, inoculation intracerebrally and intraperitoneally with 1 ml, observation period 42 days with subsequent autopsy and pathohistological examination) Beside that the vaccine was tested in nutrient media used for recovery of tubercle bacilli

In all the above cases of vaccine control quite encouraging results were obtained, indicating the absence of contamination with lymphocytic choriomeningitis virus, Coxsackie viruses monkey B virus, and tubercle bacilli

7 Residual neurotropism in Sabin's vaccine strains for Rhesus monkeys

For purposes of comparison with the author's vaccine prepared in the U S A, we studied residual neurotropism of the vaccine for Rhesus monkeys inoculated intracerebrally and intraspinally with several lots of vaccine of the three types prepared in the Institute for Poliomyelitis Research (Moscow) and in the U S A The results are given in the tables

TABLE 1 PARESIS AND PARALYSIS IN MONKEYS INOCULATED INTRACEREBRALLY WITH 1 ML OF VACCINES FROM SABIN'S STRAINS

TYPE OF VACCINE AND PLACE OF PREPARATION	UNDILUTED	1 5	
Type 1, USA	0/5		0/15
Type 2, USA	0/5		
Type 3 USA	0/5		
Type 1, USSR	0/12	0/12	0/52
Type 2, USSR	0/7	0/7	
Type 3 USSR	0/7	0/7	

In all cases intracerebral monkey test of Sabin's vaccine of Types 1, 2 and 3, and of the four lots of the vaccine prepared at the Institute for Poliomyelitis Research gave completely negative results, indicating the absence of paralytogenic activity in Sabin's attenuated strains on intracerebral inoculation of Rhesus monkeys

In all cases of paralytic disease in monkeys on intraspinal inoculation there was a benign, very mild course with complete or significant recovery of muscle function; there were no fatal cases

The data in the tables show the absence of statistically significant difference in levels of residual neurotropism on intraspinal inoculation with Sabin's original vaccine of Types 1, 2, and 3 and our vaccine, prepared from the first passage of the same strains

At the same time, these results point to the great importance of standardization of techniques of intraspinal inoculation with live virus vaccine, which was emphasized by Dr Sabin and other workers Unnecessary traumatization in the IVth segment might lead to extension of paresis and paralysis in M Rhesus on intraspinal tests of live vaccine However realizing the imperfection of our technique of intraspinal inoculation compared to that applied by Dr Sabin, we must still note the extremely benign course and outcome of paresis and paralysis in our monkeys after intraspinal inoculation with Sabin's vaccine strains This indicates the absence in vaccine strains of poliovirus of the capacity to produce generalized process, and, consequently, the significant attenuation of Sabin's strains We consider it established that, being completely innocuous on intracerebral inoculation of monkeys and absolutely apathogenic for human beings on oral administration, live vaccine from Sabin's strains, under conditions of our relatively crude methods of intraspinal inoculation, still possesses regularly manifested spinal neurotropism, producing in M Rhesus a benign disease

8 At the Institute for Poliomyelitis Research experimental studies have been successfully carried out to test the possibility of incorporation of live virus vaccine into syrup-filled bonbons, which makes it possible to improve packing, storage, and shipment of the vaccine and also to apply it more widely, using it as a pleasant product—bonbons.

9. Studies on stability of live attenuated vac

6. CHARACTERISTICS OF LIVE POLIOVIRUS VACCINE PRODUCED IN THE INSTITUTE FOR POLIOMYELITIS RESEARCH, ACADEMY OF MEDICAL SCIENCES OF THE USSR, AND COMPARISON TO SABIN'S ORIGINAL VACCINE FROM ATTENUATED POLIOVIRUS STRAINS

M. P. CHUMAKOV, A. V. GAGARINA, V. A. LASHKEVICH, S. G. DZAGUROV, N. M RALPH, G. P. FLEER, M. K. VOROSHILOVA, AND I. A. ROBINZON

Institute for Poliomyelitis Research,
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DR. CHUMAKOV (*presenting the paper*) · 1 In the Institute for Poliomyelitis Research several lots of live poliovirus vaccine were prepared in December 1958-January 1959 from Professor Albert Sabin's attenuated strains The total volume of the vaccine is enough for immunization of 10 million people (over 100 liters of each of the three types)

STRAINS USED FOR PROPAGATION

	TITER ON 21 NOV, 1958 $TCID_{50}$ PER 1 ML (cytopathogenic test)
Type 1—Lsc, 2ab	$10^{7.[illegible]}$
Type 2—P712, ch, 2ab	$10^{7.[illegible]}$
Type 3—Leon, $12a_1b$	$10^{7.2}$

These strains, which are the progeny of specially selected single plaques of the virus triply purified by successive plaque passages in monkey kidney cells under agar, were taken for propagation directly from vaccine vials (sent by Prof A. Sabin in September 1958) or from the first passage of these strains in monkey kidney cell cultures

2. Method of vaccine production is analogous to that described by Sabin in the specification for his vaccine of 10 March 1958 Vaccine poliovirus strains grew in kidney cell cultures with the medium consisting of 0 5% lactalbumin hydrolysate and 2% heated calf serum in Earle's solution gassed with CO_2 to pH 7 5. About 10% of total normal (*uninfected*) kidney cell cultures were left as controls for the absence of spontaneous cytopathogenic viruses The 28-day observation of these cultures did not show any spontaneous infection of monkey kidneys with cytopathogenic viruses Bacteriologic sterility test of vaccine viruses before filtration was made in glucosated meat-peptone broth, Kitt-Tarozzi medium and Sabouraud medium (at 37°C and 22°C)

Harvested virus-containing culture fluids were passed through plate separator ASG—3, followed by Seitz sterilizing filter pads "S" (pore diameter=0.5μ), and then distributed in 3 ml. volumes in ampules, frozen at −20° —22°C and stored until use Three ml. of vaccine contain 300 doses at 10^5 $TCID_{50}$ and over.

3 *Vaccine virus titers after filtration* were assayed by cytopathogenic test in roller kidney cell cultures (10 tubes per 0.5 log 10-fold dilution) on medium with 0 5% lactalbumin hydrolysate, 2% calf serum in Earle's solution, pH 7.5 The following titers were established for the vaccine viruses

Type 1—$10^{7.[illegible]}$ $TCID_{50}$ in 1 ml
Type 2—$10^{7.[illegible]}$ $TCID_{50}$ in 1 ml
Type 3—$10^{7.[illegible]}$ $TCID_{50}$ in 1 ml

4 Type specificity of the produced lots of vaccine from Sabin's strains was checked in neutralization tests with standard rabbit antisera (antisera in 1 · 10 and 1 : 50 dilutions and viruses in 10^{5} dilution) The results of the neutralization tests showed that all lots of live poliovirus vaccine prepared at the Institute were strictly type-specific.

5. Control for the absence in the final lots of live virus vaccine of non-poliomyelitis cytopathogenic agents was carried out by neutralization tests with undiluted virus mixed with undiluted

potent rabbit sera (with titer > 1 : 6000). In no case was any non-poliomyelitis cytopathogenic agent found in the prepared lots of the vaccine

6 Control for the absence in the vaccine of lymphocytic choriomeningitis virus was done in white mice. Control for the absence in the vaccine of Coxsackie group of viruses was carried out by inoculation of suckling white mice Absence in the vaccine of monkey B virus was tested by intracutaneous and subcutaneous inoculations of rabbits (6 rabbits per vaccine lot), and also by inoculation of monolayer rabbit kidney cell cultures Control for the absence of tubercle bacilli was done in guinea pigs (3 animals per lot, inoculation intracerebrally and intraperitoneally with 1 ml, observation period 42 days with subsequent autopsy and pathohistological examination) Beside that the vaccine was tested in nutrient media used for recovery of tubercle bacilli

In all the above cases of vaccine control quite encouraging results were obtained indicating the absence of contamination with lymphocytic choriomeningitis virus Coxsackie viruses, monkey B virus, and tubercle bacilli

7 Residual neurotropism in Sabin's vaccine strains for Rhesus monkeys

For purposes of comparison with the author's vaccine prepared in the U S A, we studied residual neurotropism of the vaccine for Rhesus monkeys inoculated intracerebrally and intraspinally with several lots of vaccine of the three types prepared in the Institute for Poliomyelitis Research (Moscow) and in the U S A The results are given in the tables

TABLE 1 PARESIS AND PARALYSIS IN MONKEYS INOCULATED INTRACEREBRALLY WITH 1 ML OF VACCINES FROM SABIN'S STRAINS

TYPE OF VACCINE AND PLACE OF PREPARATION	UNDILUTED	1 5	
Type 1, USA	0/5		0/15
Type 2, USA	0/5		
Type 3, USA	0/5		
Type 1, USSR	*0/12*	*0/12*	0/52
Type 2, USSR	0/7	0/7	
Type 3, USSR	0/7	0/7	

In all cases intracerebral monkey test of Sabin's vaccine of Types 1, 2 and 3, and of the four lots of the vaccine prepared at the Institute for Poliomyelitis Research gave completely negative results, indicating the absence of paralytogenic activity in Sabin's attenuated strains on intracerebral inoculation of Rhesus monkeys

In all cases of paralytic disease in monkeys on intraspinal inoculation there was a benign, very mild course with complete or significant recovery of muscle function, there were no fatal cases.

The data in the tables show the absence of *statistically significant difference in levels of residual* neurotropism on intraspinal inoculation with Sabin's original vaccine of Types 1, 2, and 3 and our vaccine, prepared from the first passage of the same strains

At the same time, these results point to the great importance of standardization of techniques of intraspinal inoculation with live virus vaccine, which was emphasized by Dr Sabin and other workers Unnecessary traumatization in the IVth segment might lead to extension of paresis and paralysis in M Rhesus on intraspinal tests of live vaccine However realizing the imperfection of our technique of intraspinal inoculation compared to that applied by Dr Sabin, we must still note the extremely benign course and outcome of paresis and paralysis in our monkeys after intraspinal inoculation with Sabin's vaccine strains This indicates the absence in vaccine strains of poliovirus of the capacity to produce generalized process, and, consequently, the significant attenuation of Sabin's strains We consider it established that, being completely innocuous on intracerebral inoculation of *monkeys* and absolutely apathogenic for human beings on oral administration, live vaccine from Sabin's strains under conditions of our relatively crude methods of intraspinal inoculation still possesses regularly manifested spinal neurotropism, producing in M Rhesus a benign disease

8 At the Institute for Poliomyelitis Research experimental studies have been successfully carried out to test the possibility of incorporation of live virus vaccine into syrup-filled bonbons which makes it possible to improve packing *storage, and shipment of the vaccine* and also to apply it more widely, using it as a pleasant product—bonbons

9 Studies on stability of live attenuated vac-

cine from Sabin's strains showed that it is possible to store undiluted vaccine at room temperature for 7-12 days without any loss in titer. This will help in the future to ship the vaccine by airplanes in a thawed state and use it after 10 days' storage at room temperature Until now the vaccine was stored and shipped frozen, which is sometimes difficult It is necessary further to study conditions of vaccine storage in the thawed state and possibilities of application of chemical stabilizers for heat-stability of vaccine viruses.

10 As a result of comprehensive investigations of a large lot of live virus vaccine (for 10 million people) prepared at the Institute for Poliomyelitis Research, Academy of Medical Sciences, USSR, it has been found that this vaccine, by all its qualities, is not inferior to the author's vaccine and fully corresponds to the requirements of the Expert Committee on Poliomyelitis, WHO, for live vaccines applied to human beings

Up to the present this vaccine was used for immunization of over 1,500,000 people in a number of republics and regions of the Soviet Union, with very good results, indicating the complete safety, areactivity and high immunologic efficacy of this preparation.

The vaccine in 1:10 dilution was administered orally 2 drops in a spoonful of tea, water or on a piece of cake, both as separate monovaccines (Type 1 → Type 3 → Type 2) and bivalent (Types 1 + 3) and trivalent mixtures. The minimal amount of vaccine virus was not less than 100,000 TCID$_{50}$ and the maximum was about 700,000 TCID$_{50}$

We are sure that decisive victory in elimination of poliomyelitis epidemics will probably be connected with large-scale application of live attenuated virus vaccines having some advantages over Salk killed vaccine

TABLE 2 PARESIS AND PARALYSIS IN M RHESUS MONKEYS INOCULATED INTRASPINALLY INTO THE LUMBAR ENLARGEMENT WITH 0 1 ML. OF LIVE VIRUS VACCINE FROM SABIN'S STRAINS

Vaccine Type and Place of Preparation	Virus Titer Log_{10} TCD_{50}	Number of Monkeys Paralyzed								% Clin Involvement
		Undil		10^{-1}		10^{-2}		10^{-3}		
		Clin	Hist	Clin	Hist	Clin	Hist	Clin	Hist	
Type 1, USA	7 2	8/8	8/8	3/8	6/7	1/8	5/8	1?/8	4/8	40 6
Type 2, USA	7 4	1+1?/6		1+1?/6		4/6		2+1?/6		41 6
Type 3, USA	7 2	2?/6		1+1?/6		2/6		1?/5		30 4
Type 1, USSR*	7 8	5/7	4/4	5/7	6/7	6/7	6/7	2/6	2/6	66 6
Type 1, USSR**	7 6	3+2?/5		1+2?/6		3/6		2/5		56 5
Type 2, USSR	7 8	2/6		2/6		3/6		1/5		34 7
Type 3, USSR	7 2	4/6		2/6		2/6		0/6		33 3

* Before distribution into ampules
** Vaccine from ampules
1?/8—One of the 8 monkeys had traumatic paresis seen within 24 hours after inoculation, with subsequent limited extension

Table 3 Characteristics of Paresis and Paralysis in Rhesus Monkeys after Intraspinal Inoculation with Sabin's Vaccine Strains

Dilution and TCD_{50} in 1 0	Paresis	Dr Sabin's Experiments			Our Experiments						
					With Sabin's Original Vaccine			With Our Vaccine			
		No 1 Type 1	No 2 Type 1	No 3 Type 1	Type 1	Type 2	Type 3	Type 1	Type 1	Type 2	Type 3
Undil 6 2—6 8	Sl	—	—	2	3	—	—	2	1	—	2
	M	—	—	2	5	1	—	3	2	2	2
	?	1	1	—	—	1	2	—	2	—	—
	N	9	9	6	—	4	4	2	—	4	2
		10	10	10	8	6	6	7	5	6	6
10^{-1} 5 2—5 8	Sl	—	1	—	2	—	1	2	—	—	1
	M	—	—	3	1	1	—	3	1	2	1
	?	1	2	—	—	1	1	—	2	—	—
	N	4	2	2	5	4	4	3	3	4	4
		5	5	5	8	6	6	8	6	6	6

10^{-4} 4.2–4.8	Sl	—	—	—	1	1	2	3	1	—	—
	M	—	—	—	—	2	—	3	2	3	2
	?	1	—	—	—	—	—	—	—	—	—
	N	4	5	5	7	3	4	1	3	3	4
		5	5	5	8	6	6	7	6	6	6
10^{-3} 3.2–3.8	Sl	—	-	—	—	2	—	1	—	—	—
	M	—	—		—	1	-	1	2	1	—
	?	—		—	1	1	1	—	—	—	—
	N	—	—	-	7	2	4	4	3	4	6
		—	—	—	8	6	5	6	5	5	6
		3?/20 15%	4?+3/20 35%	8/20 40%	12+13/32 40.6%	8+3?/24 45.82%	3+12/23 30.4%	18/28 64.3%	9+4?/22 59.9%	8/23 34.8%	8/24 33.3%

Sl — Mild limited paresis with recovery of function
M — Moderate paresis or paralysis of extremity with incomplete recovery
? — Localized extension of traumatic paralysis seen within 24 hours
N — Normal animals

TABLE 4 PARALYTOGENIC EFFECT IN RHESUS MONKEYS INOCULATED WITH VACCINE FROM SABIN'S STRAINS

VACCINE TYPE	TITER IN 1 ML	INTRACEREBRALLY 1 ML		INTRASPINALLY 0 1 ML			
		UNDILUT	1 5	UNDIL	10^{-1}	10^{-2}	10^{-3}
Type 1, USA	7 2	0/5		8/8	3/8	1/8	1?/8
Type 2, USA	7 4	0/5		1+1?/6	1+1?/6	3/6	2+1?/6
Type 3, USA	7 2	0/5		2?/6	1+1?/6	2/6	1/6
Type 1, USSR	7 6	0/12	0/7	3+2?/5	1+2?/6	3/6	2/6
Type 2, USSR	7 8	0/7	0/7	2/6	2/6	3/6	1/5
Type 3, USSR	7 2	0/7	0/7	4/6	2/6	2/6	0/6

Note Numerator—number of monkeys with paresis and paralysis
Denominator—number of monkeys inoculated
1?/8—number of monkeys with involvement seen within 24 hours after inoculation (extension of traumatic paresis)

Table 5 Paralysis and Paresis in M Rhesus Monkeys after Intraspinal Inoculation Virus V Vaccine from Sabin's Strains

Dilution	TCD_{50} in 0 1 ml	No of Monkeys Paralyzed							
		Dr Sabin's Experiments			Our Experiments				
		No 1 Type 1	No 2 Type 1	No 3 Type 1	Type 1 USA	Type 1 USSR	Type 1 USSR	Type 2 USSR	Type 3 USSR
Undil	6 2-8 8	1?/10	2+2?/10	2+3?/10	8/8	5/7	1+2?/5	2/6	4/6
10^{-1}	5 2-5 8	1?/5	1+2?/5	3/5	3/8	5/7	1+2?/6	2/6	2/6
10^{-2}	4 2-4 8	1?/5	0/5	0/5	1/8	6/7	3/6	3/6	2/6
10^{-3}	3 2-3 8			0/4	1?/8	2/6	2/6	1/5	0/6
		3?/20 15%	7/20 35 0%	3+4?/20 40 0%	13/32 40 6%	18/27 66 6%	13/23 56 5%	8/23 34 0%	8/24 33 3%

Note Numerator—number of monkeys paralyzed 2 days after inoculation
Denominator—number of monkeys inoculated and surviving for 14 days
? —involvement only of toes or localized extension of traumatic paralysis first seen within 24 hours after inoculation and associated with focal poliomyelitis lesions insufficient to account for clinical paralysis

DISCUSSION

CHAIRMAN ANDERSON Thank you very much, Dr Chumakov

The papers of Dr Murray, Dr Kirschstein, Dr Melnick, Dr. Cabasso, Dr. Koprowski, and Dr Chumakov are now open for discussion

Dr Paul

DR PAUL I would like to discuss some aspects of the first three papers, by Dr Murray, Dr Melnick, and Dr Koprowski

The first two reflect a tremendous amount of work in testing neurovirulence in the monkey and as a marker of the poliovirus strains I think that we are fortunate to have seen these results, when we realize the extent of the work that is necessary in order to make this sort of determination

Dr Koprowski has pointed out that neurovirulence as measured in the monkey is not the only marker He has measured two or three more, and I suspect that there are probably more than have been mentioned today I refer to the capacity of strains to produce viremia How that is to be measured I am not sure, but I suspect that it could be done in primates and also in vaccinees

But a third capacity of these poliovirus strains is the one that Dr Dick mentioned earlier today This is the *stability* of the strain It would seem to be a crucial measurement to be made in the selection of an attenuated poliovirus for use in a vaccine Whether Dr Lwoff's new technique in subjecting these strains to different temperatures would give one a measure of stability is a point that is well worthy of discussion at this meeting

I mention these things merely in an effort to interpret what we have heard today

CHAIRMAN ANDERSON · Dr Sabin

DR SABIN · I hope that I may be permitted to take some time to discuss the difficult problem of evaluation of the quantitative aspects of neurotropism by different techniques In the work that I have been doing on attenuated polioviruses, I have inoculated every monkey myself (by now over 10,000), both intracerebrally and intraspinally Insofar as at least one individual can be constant, and his hand is not always constant, I have a certain amount of accumulated experience, because I have also personally checked the histologic findings.

In the first place, I would like to say that when I returned from Moscow I brought back samples of the large lots that were grown in Dr Chumakov's laboratory and in Dr. Smorodintsev's laboratory. I have recently completed tests on these by the intraspinal technique that I have used all along, and at the same time rechecked my original Type 1 vaccine that served as the seed virus for their lots I have obtained results entirely comparable to those that Professor Chumakov has reported

But there was one problem that troubled me That was that in previous tests with the Type 1 vaccine that I had used, inoculating it at the 10^{-2} dilution, which contains about 10^{5} TCD_{5} out of 24 monkeys inoculated at different periods of time, there were only 2 in whom some weakness had been observed

But in testing the Russian large lots there were 6 out of 10 monkeys that exhibited for the most part only weakness of one extremity

I then tested the original vaccine in the same lot of Rhesus monkeys that I had used for these tests Because I thought that the precise distance which the needle is withdrawn after hitting the ventral bony surface might make a difference, I inoculated two groups—one in which I left the needle rather close to the bone, hoping to hit most of the ventral portion of the anterior horn, and the other in which I withdrew about 2 mm as usual It turned out that more involvement clinically was apparent in the latter group—and the results this time were comparable to the ones I obtained with the large Russian lots *It is possible, therefore, that some* variation in results may also be due to differences in different groups of monkeys

But I am impressed by the fact that the differences that I obtained were not between no paralysis or slight weakness and extensive

paralysis of one or both extremities, but rather, between no paralysis and a partial, usually non-progressive involvement

This brings me to the point of how much is actually left in the spinal gray matter with this technique that I have used for years I know that Dr Melnick is sometimes inclined to make flippant remarks, and in referring to this technique he said that he did not want merely to "wet" the spinal cord with virus, but instead to put in the whole inoculum

In the first place, I am sure that Dr Melnick is fully aware of my previously published data which showed that, with viruses that were less attenuated, it was possible to dilute them out to 10^{4} and 10^{5} and still obtain extensive paralytogenic activity by the technique that I use

I know that he is also aware of the fact that the same strains provided to him by the Lederle Laboratories were also tested in my laboratory jointly with Dr. Cox and Dr. Cabasso

My method of spinal inoculation gave precisely the same end point of extensive paralytogenic activities as that obtained by Dr Melnick and by Dr Murray It is evident, therefore, that by the method that I use a better differentiation can be obtained in spinal neurotropism.

Much has been said here about spread beyond the cord I think we must interpret "spread" very carefully Dr Melnick has seen the sections from many of the monkeys that I have inoculated, and he knows that by my method of inoculation there is a lesion in the anterior horn, which destroys only a part of the anterior horn, but does not extend over many levels of the spinal cord, which is usual for the method that he uses

From tests that I have carried out myself with India ink, using very small and large gauge needles, and from tests performed by others, I know that it is possible by intraspinal inoculation, *particularly with the small needle, to pro*duce occasionally such a jet stream that the India ink not only spreads outside of the spinal cord, along the pia arachnoid space all the way up to the medulla as it does regularly, but also through the gray and white matter directly up through the thoracic and cervical cord to the medulla

Under such conditions, one can speak of the spread of the inoculum, but not of the spread of the virus by propagation from one infected neuron to another

Now, Dr Melnick may say. "But I can show you many monkeys inoculated by such jet stream and no lesions at all were found in the cervical cord" That is quite true If a particular virus does not readily multiply at the inoculated site with the small amounts that may reach the cervical cord, etc, you get no lesions or very few lesions. If it does multiply readily, then you can get not only lesions but also paralysis

Furthermore, what is the significance of involvement of both extremities, when Dr Melnick takes his needle and moves it around in many different directions during the course of the inoculation?

Under these conditions one is obviously not measuring the spread of the virus due to progressive multiplication but rather the spread of the inoculum, i e, of the initial mechanical dissemination, assuming that injured neurons may not actually be more sensitive to virus or injured tissue to extraneural multiplication Dr Melnick has here a very excellent method for determining the activity of viruses that may be completely without the capacity to multiply in monkey neurons That is fine, but there are no such viruses at the present time Even the most attenuated, avirulent 25°C. mutant that we have been able to get does not belong to this category

Dr Cabasso has raised the question of whether the spinal test can be used as a practical means of checking on the neurotropic activity of different strains or different lots of the same strain

I think that it can and should be so used If in scoring the results the differences between no paralysis, weakness or partial paralysis on the one hand and severe, extensive unilateral and bilateral paralysis on the other—with a technique in which the inoculation is left at one level of the lumbar cord (not moving the needle around and not injecting it in a jet stream)—then my experience shows that valuable information can be obtained

I think that all the strains that have been described here are attenuated, and there is no doubt from the data presented by Dr Melnick and Dr Murray, as well as from my own, that there are differences among them What we want to do, it seems to me, with all these excellent and laboriously gathered data is to first

of all find out whether or not there is law and order in this

I think there is law and order. One of the striking pictures that was shown this afternoon by Dr Cabasso indicated that a large concentration of one of his viruses could be inoculated within a millimeter or less away from the matter of the spinal cord and not produce even lesions, while a minute fraction of this dose placed in the anterior horn produces paralysis I can confirm this

All these data are of the greatest importance in telling us what to do in the future for biologic assay and control of such a vaccine They are of tremendous help, but let us not be left with the impression that it is all confusion I think there is law and order in all of this

CHAIRMAN ANDERSON Dr Melnick

DR MELNICK *I would like to continue along* the line of Dr Sabin's discussion, particularly in view of his mentioning the section which Dr. Cabasso showed on the screen This is an excellent example of showing where a needle went through the cord, but, as I remember the section I saw no evidence of material having been *deposited in that particular area The crucial* point is Is it enough to have evidence of the needle penetration damage, or should there be some evidence that the inoculum has been left behind in the spinal cord?

CHAIRMAN ANDERSON Dr Sabin

DR SABIN I have taken the trouble to measure the volume of the lumbar enlargement of the spinal cord and it is only about 0 5 to 0 7 ml If all of this 0 1 ml. were to be left in there, you would blow out the entire anterior horns into the subarachnoid sphenoid space—and you often do

So, whatever method of *inoculation you use* you do not have all of the material left in the anterior horn

The fact that there is enough material left after putting the inoculum in one site so that when the virus is active one can inoculate only 10^2 TCD_{50} or less and still produce paralysis, is evidence of *the effectiveness* of a method—provided the inoculum is in the gray matter in or near the anterior horn.

It cannot be said that by any method all of the material remains in the gray matter. I agree that by the method that Dr. Melnick has used more of the material remains, but at what expense? At the expense of knocking out a large portion of the anterior horn cells mechanically so that when you add on top of that a limited amount of virus action, you have the straw that breaks the camel's back.

DR MELNICK It seems to me that we are looking for the most sensitive method for detecting virus growth in the lumbar cord and one which readily allows one to discriminate between strains of different degrees of attenuation To me Dr Sabin seems to be saying that we should not use the most sensitive method, but one that will not detect differences among his three attenuated strains

DR SABIN That is not what I am saying, the point that I am making is that with the most sensitive method that you have described, and particularly if you merely count weakness and partial paralysis, and if you give that the same weight as extensive, complete paralysis, involving more than one extremity quantitatively, you cannot distinguish between two strains which have a great deal of difference between them Whereas, by using a method in which you place the inoculum at one point of the lumbar cord, and you allow it to multiply there, and then to spread subsequently by growth, you can distinguish between strains which by your method, which is so sensitive, you cannot distinguish

CHAIRMAN ANDERSON Dr Murray has the floor

DR MURRAY: *I think a very important principle* in any biological assay of activity is that of measuring the degree of response, and I think that *rather than trying to get* something which is less sensitive and therefore proving, perhaps, to be the thing which you do not want to get, that the important point is to develop a method which is not only sensitive, but is capable of discriminating between different strains This I do believe is what we have achieved in the various methods which have been used for spinal inoculation.

I think this shows up in the results which we exhibited this morning, and it also shows up in

the results which Dr. Melnick has exhibited It also shows up in Dr Sabin's results.

The thing which is perhaps more crucial, and I have noticed that Dr Sabin did not allude to it, is the differences between the intracerebral inoculation and the intraspinal inoculation Here also, matters of technique are of extreme importance and we have evidence which indicates that *unless* inoculations are *made into* the thalamus, the results are negative

We would be glad to show a slide illustrating this, if there is a moment for it

Chairman Anderson Is the slide ready?

Dr Sabin While this slide is being shown, may I add that Dr. Murray has stated that I did not allude to the proper placement of an intracerebral inoculum From my own experience, if the inoculum is not put into a susceptible area, as I previously reported, you may get negative results, and that may account for some of the differences that have been recorded here today following intracerebral inoculation

Chairman Anderson Dr Kirschstein will present the slide

Dr Kirschstein In this table (Table 13)[1] in the column entitled "Intracortical" inoculation in the animals in this group the brains were cut for evidence of inoculation trauma The inoculum was found to go for the most part, either into the cingulate gyrus the corpus callosum, or occasionally the lateral ventricle

In the column entitled Intrathalamic" it was found to go into the thalamus on both sides

In Type 1 material, when the inoculum was placed intracortically, in this fairly haphazard way, only 2 out of 5 monkeys inoculated with the undiluted material showed lesions

When the inoculum was placed in the thalamus, monkeys were found to come down with lesions all the way down to the 10^{-4} dilution

In the Type 3, the results were perhaps even more striking in that no animals came down with lesions at any dilution, including the undiluted material when the inoculum was placed haphazardly and at least one animal came down at the 10^{-3} dilution when the inoculum was placed intrathalamically.

Chairman Anderson Dr Bodian

Dr Bodian I would like to comment on something that has not been referred to, perhaps because it is so obvious. But I think it is more important than some of the discussion of details of method These details are problems of standardization that can easily be straightened out in time

It is important that Dr Murray has presented unique data dealing with the testing, by a standard method, of strains of different origins It seems to me that we should look at this rather carefully The results show, when a single method is used by experienced people, that there are striking differences among strains which have already been used in the field, some being more neurovirulent than others

Incidentally, this finding clarifies inconsistencies of reports concerning these strains made by different laboratories testing these strains We now have one laboratory which has tested strains of all three types from the three sources

I would now like to ask Professor Chumakov what his interpretation would be of the differences shown in intraspinal virulence of the Type 1 strains which they have produced, and the original Type 1 material supplied by Dr Sabin As I see it, in the first column there is an attack rate of 15 per cent or so in Dr Sabin's experience, and no less than 40 6 per cent in each of three runs with Moscow material Is this to be considered a difference in technique of inoculation? Or, is this a difference due to change in virulence? What is your feeling about this Professor Chumakov?

Chairman Anderson Dr Chumakov

Dr Chumakov (through an interpreter) The primary difference I am sure, is technical because of a much more crude technique that was used as compared to Dr Sabin's

The statistical differences in this case may emphasize or present apparent differences in what actually, in both experiences amounted to apparently a virulent, safe vaccine

There is another possible source of difference in the results even in our own experience the

[1] See p 52

of all find out whether or not there is law and order in this

I think there is law and order One of the striking pictures that was shown this afternoon by Dr Cabasso indicated that a large concentration of one of his viruses could be inoculated within a millimeter or less away from the matter of the spinal cord and not produce even lesions, while a minute fraction of this dose placed in the anterior horn produces paralysis I can confirm this.

All these data are of the greatest importance in telling us what to do *in the future for biologic* assay and control of such a vaccine They are of tremendous help, but let us not be left with the impression that it is all confusion I think there is law and order in all of this

CHAIRMAN ANDERSON Dr Melnick

DR MELNICK I would like to continue along the line of Dr Sabin's discussion, particularly in view of his mentioning the section which Dr Cabasso showed on the screen This is an excellent example of showing where a needle went through the cord, but, as I remember the section, I saw no evidence of material having been deposited in that particular area. The crucial point is Is it enough to have evidence of the needle penetration damage or should there be some evidence that the inoculum has been left behind in the spinal cord?

CHAIRMAN ANDERSON: Dr Sabin

DR SABIN I have taken the trouble to measure the volume of the lumbar enlargement of the spinal cord and it is only about 0 5 to 0 7 ml If all of this 0 1 ml were to be left in there, you would blow out the entire anterior horns into the subarachnoid sphenoid space—and you often do

So, whatever method of inoculation you use, you do not have all of the material left in the anterior horn

The fact that there is enough material left after *putting the* inoculum in one site so that when the virus is active one can inoculate only 10^2 TCD_{50} or less and still produce paralysis, is evidence of the effectiveness of a method—provided the inoculum is in the gray matter in or near the anterior horn.

It cannot be said that by any method all of the material remains in the gray matter I agree that by the method that Dr Melnick has used more of the material remains, but at what expense? At the expense of knocking out a large portion of the anterior horn cells mechanically so that when you add on top of that a limited amount of virus action, you have the straw that breaks the camel's back.

DR. MELNICK: It seems to me that we are looking for the most sensitive method for detecting virus growth in the lumbar cord and one which *readily allows one to discriminate between strains* of different degrees of attenuation To me Dr Sabin seems to be saying that we should not use the most sensitive method, but one that will not detect differences among his three attenuated strains

DR SABIN. That is not what I am saying; the point that I am making is that with the most sensitive method that you have described, and particularly if you merely count weakness and partial paralysis, and if you give that the same weight as extensive, complete paralysis, involving more than one extremity quantitatively, you cannot distinguish between two strains which have a great deal of difference between them Whereas, by using a method in which you place the inoculum at one point of the lumbar cord, and you allow it to multiply there, and then to *spread subsequently by growth, you can* distinguish between strains which by your method, which is so sensitive, you cannot distinguish

CHAIRMAN ANDERSON Dr Murray has the floor

DR MURRAY: I think a very important principle in any biological assay of activity is that of measuring the degree of response, and I think that rather than trying to get something which is less sensitive and therefore proving, perhaps, to be the thing which you do not want to get, that the important point is to develop a method which is not only sensitive, but is capable of discriminating between different strains This I do believe is what we have achieved in the various methods which have been used for spinal inoculation

I think this shows up in the results which we exhibited this morning, and it also shows up in

differences in this respect between different lots of monkeys, and I think that is a very reasonable supposition

In the tables presented you will find that dose response curves have extremely flat slopes (Fig 2) I remember particularly one table presented by Dr Murray, where 2 out of 5 monkeys came down in four consecutive tenfold dilutions On account of the flat slope of the close response curves 50 per cent end points provide by no means reliable estimates of the activity In such cases, the vertical distance between two response curves is probably a more realistic expression of virulence differences than the distance between 50 per cent end points

For that reason I think the method Dr Murray used to compare different strains is not truly representative of their neuropathogenic activity

As I see it, all strains discussed so far are attenuated viruses There are certain differences between them but I would not consider those differences to be sufficient to discriminate on that basis between the various strains discussed

CHAIRMAN ANDERSON Dr Dick

DR DICK As most people know I am much more interested in what comes out, and we will be discussing this tomorrow, but I would like to emphasize what Dr Bell said by asking you to consider how you are certain that the CHAT strain did not change to whatever it was in the individual. How are we certain of that? That is the first question I would like to ask

The second question is: What do the statisticians think about tests in three rabbits, to show the absence of B virus in 16 to 20 liters of vaccine?

CHAIRMAN ANDERSON. Dr *Koprowski*

DR KOPROWSKI: I have shown that the MS marker of the CHAT strain has been retained in the course of at least three passages through human intestinal tract I have also shown that none of the Leopoldville viruses isolated during an epidemic had the MS character In addition the "serologic" marker of McBride was different for the wild strain and the attenuated virus Dr Dick is asking "How are we certain that the CHAT strain did not change to whatever it was in the individual?" Here are scientific facts showing that a study of two genetic markers failed to indicate changes in the CHAT strain

If the "serologic" marker will prove to be a stable character of polio virus then I believe we will have quite a useful tool in tracing the antigenic changes of polio virus, if any will occur, after passages through man

This study of genetic markers mentioned in my paper and of other markers referred to by Dr Paul and other participants may in time supplant the use of monkeys After listening to the excellent and enormous work conducted in

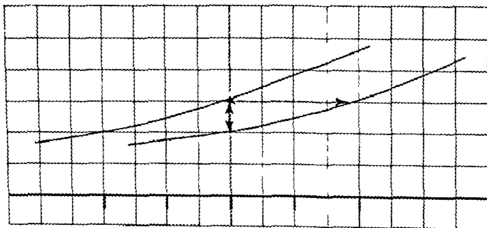


FIG 2 Dose Response Curves of Attenuated Strains

same lot of vaccine, shown in the first two columns under our experiments, gives a considerable variation under identical conditions However, the conditions were prior to ampuling of the vaccine; after ampuling, it showed a difference between 40 6 and 66 6 per cent

I feel that possibly the difference in the titers prior to and after distribution of vaccine to the ampules may conceivably—I do not state that this is so—but may be another factor introducing a difference.

Chairman Anderson Dr Sabin

Dr Sabin: The tables to which Dr Bodian refers do not include the simultaneous tests that I have carried out recently As I said a little while ago, in the same lot of monkeys the original vaccine and the tertiary lot produced in Dr. Chumakov's laboratory yielded comparable results when I inoculated all the monkeys

In Dr Chumakov's laboratory, Dr Ralph, who inoculated the monkeys, has followed essentially the procedure of Dr Melnick, using a very fine needle and multiple placement of the inoculum The results obtained in Moscow are very comparable to those that Dr Murray reported, and somewhat comparable to those that Dr Melnick has obtained

Furthermore, to show the role of technique again, Dr Smorodintsev tested the 20 liter lots that he produced, and monkeys that had been inoculated He got completely negative results on intraspinal inoculation, and it appeared at first they were quite different from the lots that Dr Chumakov's laboratories prepared When I tested them simultaneously, I found no difference between the lots produced by Dr. Smorodintsev and the lots produced by Professor Chumakov

Chairman Anderson: Dr. Gard

Dr Gard I do not feel competent to enter into the discussion of the very refined technique for determination of neurovirulence, but I would like to say a few words about evaluation of the results obtained In order to make my point clear, I would like to use the blackboard

A virulent virus gives a dose response curve of the shape shown in Figure 1 below The 50 per cent end point represents a reliable measure of the amount of virus present in your material, and comparing two preparations, the distance between the 50 per cent end points represents a good measure of the difference between the two specimens

When measuring neurovirulence of attenuated viruses, we are far from this ideal situation One virus particle does not produce paralysis, except under very special circumstances and we must suspect that the decisive factor is the *susceptibility of the particular animal inoculated* Dr Sabin mentioned the possibility of

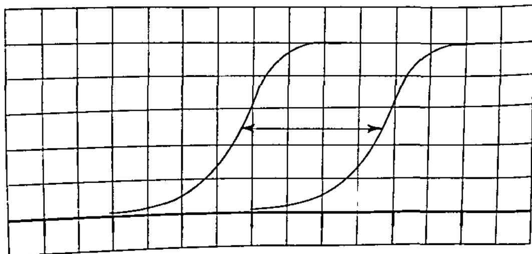


Fig 1 Dose Response Curves of Virulent Strains

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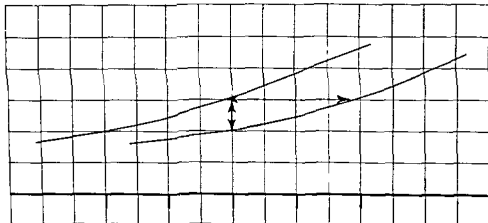


FIG 2 Dose Response Curves of Attenuated Strains

monkeys with attenuated polio viruses. I believe that the time has come when we should somehow declare a moratorium on the use of monkeys for large-scale tests With presently available strains we have "squeezed out" of monkeys all information it is possible to obtain

Perhaps in the nearest future we should study, first of all, the "laboratory" markers of a strain or its variant before indulging in the luxury of the use of hundreds or thousands of monkeys.

In passing, I would like also to voice my objection to the use of the term "neurovirulence" in reference to the character of a virus I would like to emphasize what has already been mentioned by Dr. Gard, that virulence is an interplay phenomenon between the infectious agent and its host and, that we should not forget that the host exists

Dr Dick has asked me if the serologic marker of the CHAT strain and that of Sickle strain are the same We do not know the serologic characteristics of the Sickle virus or its other genetic markers We shall study these problems in the nearest future

Chairman Anderson Dr Gear

Dr Gear We have carried out a number of tests using the South African monkey, and I thought it might be of some interest to present briefly the results of the tests done by Dr Winter with Dr Sabin's strain

The Type 1, LSc 2ab strain was inoculated intracerebrally into 4 monkeys, each receiving 0.5 ml. into the thalamic region Of the 4 monkeys inoculated, none showed paralysis; histologically one showed a thalamic focus and one a cervical focus of inflammation

Of 5 monkeys inoculated intraspinally, one developed paralysis and the others all recovered from the initial leg weakness Histologically all showed lesions in the lumbar cord, indicating proper placing of the inoculum Two monkeys showed extension of these lesions one to the region of the fourth ventricle and one to the cervical cord

Viremia tests were all negative on the fifth day Of the cord cultures, one was positive on the 22nd day, when the monkeys were killed Only one developed antibodies. The others were negative

The Type 2, P712-2ab strain was given intracerebrally. Of 6 monkeys inoculated into the thalamic region, none developed paralysis. Histologically foci were seen in the thalamus and in the cortex in 4 monkeys; 2 showed no lesions In one monkey, there was extension to the region of the fourth ventricle, and a focus in one anterior horn of the lumbar cord.

Five monkeys were inoculated intraspinally Apart from weakness in one monkey lasting from the 14th to the 18th day, no paralysis occurred. Histologically all showed foci in the lumbar cord. In 2 monkeys the lesions were confined to the lumbar cord In the third there was extension to the thoracic region In the remaining two, there was greater extension, the lesion involving the cervical cord, the fourth ventricle and thalamic areas In the other, foci were found in the medulla and fourth ventricle area Virus was recovered from the cord of this monkey. Viremia tests showed one positive on the 5th day Of the cord cultures, one was positive. In the antibody tests, two were positive

The Type 3 virus, Leon 12ab strain was inoculated intracerebrally. Of 5 monkeys, apart from some early weakness, none showed paralysis Histologically, 2 monkeys showed a slight reaction in the thalamus only The others were clear.

Four monkeys were inoculated intraspinally *Two showed paralysis Histologically, all* showed lesions in the lumbar cord One showed lesions throughout the central nervous system One had foci in the medulla in addition to the lumbar region The remaining two showed lumbar cord lesions only None showed viremia The cord cultures were negative, and five developed antibodies

One conclusion we draw from these results is that the South African vervet monkey is probably suitable for these tests.

Chairman Anderson Dr. Bodian

Dr Bodian I would like to comment briefly on Dr Gard's interpretation of the results of the tests on neurovirulence I cannot entirely agree with his interpretation, because I think we are dealing with a comparison of neurovirulence of strains which appear above the base line in the one case, and on the other, of strains, the curve of which we cannot say anything about because it

is below the base line We do not know the shape of the curve for the intrathalamic inoculation of the Sabin strain

Also, I think that the differences shown by Dr Gard between the sigmoid curve and the straight line relationships are not significant, because it would take a very large number of monkeys to show that the straight lines are not truly sigmoid

Worse than this, I think that we are confronted with a rather dangerous situation in relation to control if we assume that we are unable to distinguish degrees of virulence by means of the intrathalamic tests How then are we to distinguish between some of the attenuated strains we have seen and wild occurring strains of low virulence?

We will have to have a test which discriminates between vaccine viruses and contaminant viruses of low virulence

So I merely want to state, without disagreeing totally with Dr Gard, that we should not ignore differences such as those presented by Dr Murray Now, differences of this order may or may not be significant for safety, but I think we have to look at this rather carefully.

CHAIRMAN ANDERSON Dr. Gard

DR GARD I just want to remark that, of course dose response curves are sigmoid in all cases, but it will take, as Dr Bodian says, an enormous number of monkeys to detect their true and exact shape As to the actual position of dose response curves, it apparently depends upon who is doing the investigation Dr Melnick certainly found both curves above the base line in those two Type 1 strains he compared

CHAIRMAN ANDERSON Dr. Cox.

DR COX In the Andes experience, in Colombia this past year, Dr da Silva sent us stools from a number of paralytic cases and contacts that occurred in the Andes village and adjacent area One stool was obtained from a boy, about three and a half years old, who was severely paralyzed in both legs early in January 1958 The stool specimen was not obtained until the latter part of March, at least sixty days after the boy became paralyzed The stool specimen was titrated and found to contain about 50 tissue culture units of Type 1 poliovirus per gram The first tissue culture passage isolated titrated $10^{8.3}$ tissue culture doses (two hundred million) per cc The first tissue culture passage material was serially diluted in 10-fold dilutions and each dilution was inoculated intracerebrally into two monkeys, using a 1 0 cc of inoculum per monkey This strain paralyzed or produced definite clinical reactions in at least one of the two monkeys inoculated with each of the virus dilutions tested from $10^{8.3}$ through $10^{0.3}$ tissue culture doses The dilutions containing $10^{5.3}$, $10^{6.3}$, and $10^{7.3}$ tissue culture doses each paralyzed two of the two monkeys inoculated. In other words, as little as 2 tissue culture doses paralyzed one of two monkeys inoculated

This is the most virulent, or the "hottest" strain of poliovirus that I know of It is interesting to note that in the same home a sibling about three months of age was found to be excreting a Type 1 virus at the same time The stool specimen was found to contain $10^{4.7}$ (50 000) tissue culture doses per gram The first tissue culture passage material which contained $10^{8.4}$ (250 million) tissue culture doses was likewise serially diluted in 10-fold dilutions and each dilution was inoculated intracerebrally into two monkeys using 1 0 cc of inoculum for each monkey Those dilutions containing $10^{1.4}$, $10^{2.4}$, $10^{3.4}$, $10^{4.4}$, $10^{8.4}$ and $10^{7.4}$ tissue culture doses of virus paralyzed at least one monkey of the two inoculated The dilutions containing $10^{0.4}$, $10^{5.4}$, and $10^{6.4}$ tissue culture doses of virus failed to paralyze any of the inoculated monkeys This child was not paralyzed, although he presumably was infected with the same strain of virus that paralyzed his older brother

In trying to get further information in an effort to solve our problem about what degree of attenuation is necessary to have safe and satisfactory oral poliomyelitis vaccines, I thought you would be interested to know of the highly virulent properties of these two naturally occurring Type 1 strains that were isolated in the course of the studies in Andes, Colombia

CHAIRMAN ANDERSON Thank you, Dr Cox. If there are no further comments, we will close the discussion for the day

SECOND SESSION

TUESDAY, 23 JUNE 1959

Chairman

DR CHARLES H STUART-HARRIS
Professor of Medicine
University of Sheffield
Sheffield, England

TOPIC II. CRITERIA OF ATTENUATION, DEVELOPMENT, SELECTION, AND TESTING OF POLIOVIRUS STRAINS FOR USE IN FIELD TRIALS *(continuation)*

Presentation of Papers by:

Dr Hilary Koprowski

(DISCUSSION)

TOPIC III. PROPERTIES AND BEHAVIOR OF ORALLY ADMINISTERED ATTENUATED STRAINS

Presentation of Papers by:

Dr Matilda Benyesh Melnick

(DISCUSSION)

Dr Henry M Gelfand
Dr John R. Paul

(DISCUSSION)

TOPIC IV. THE PROBLEM OF INTERFERENCE IN LIVE POLIOVIRUS IMMUNIZATION

Presentation of Papers by:

Dr Herald R Cox
Dr Konald A. Prem

(DISCUSSION)

Dr Matilda Benyesh-Melnick
Dr James H. Hale

(DISCUSSION)

TOPIC II. CRITERIA OF ATTENUATION, DEVELOPMENT, SELECTION, AND TESTING OF POLIOVIRUS STRAINS FOR USE IN FIELD TRIALS *(continuation)*

7. BEHAVIOR OF ATTENUATED STRAINS OF POLIOMYELITIS VIRUS IN RELATION TO AGE, FAMILIAL SPREAD, AND DURATION OF IMMUNITY

HILARY KOPROWSKI, STANLEY PLOTKIN, JOSEPH PAGANO, THOMAS W. NORTON, AND JOSEPH STOKES, JR

The Wistar Institute of Anatomy and Biology and the Children's Hospital (J S) of Philadelphia, Pennsylvania

DR KOPROWSKI *(presenting the paper)* The country of El Dorado is an ancient patrimony of the Incas, whose ancestors prudently did not emigrate to the neighboring country of Peru in order to be destroyed there by Pizarro and his forces, but remained in their almost inaccessible domain and vowed that none of the inhabitants of their little kingdom should ever quit it When Cacambo, one of two visitors who inadvertently wandered into El Dorado from Europe, asked one of the ancient citizens of the country whether there were "among them persons to dispute, to confirm, to intrigue and to burn people who are not of the same opinion with themselves" he received a scathing reply "Do you take us for fools? Here we are all of one opinion and know not what you mean by your question " This wholesome attitude and the evidence of the general happiness of the people made El Dorado an appropriate choice for mass immunization against poliomyelitis with a living attenuated virus This was carried out among the susceptible population of children and adolescents and when it had been successfully completed the El Doradians were faced with a dilemma How were they to maintain a healthy, happy population, each member immune to polio, in the face of the high birth rate? The idea occurred to them that all newborn babies should be immunized at birth but they needed to know how such young infants would react to oral immunization They appealed for help to the United States Public Health Service and were informed that Dr Joseph Stokes, Dr Plotkin, and Dr Koprowski had recently studied the effect of live oral vaccine on infants less than 6 months old[1]

Table 1 summarizes data obtained in a study conducted in this age group It will be observed that the ratio of successful immunization in infants 0-60 days after birth is significantly lower than the ratio obtained with infants two months of age or older, or with children and adolescents. Since transplacental antibodies were present in the majority of infants who were fed virus, the effect of this antibody upon virus feeding had to be investigated Results summarized in Table 2 indicate that the slightly lower susceptibility of very young infants to intestinal infection with attenuated polio virus cannot be explained by the relatively high level of transplacental antibodies, commonly present in the newborn Infants who fail to be immunized after a first feeding of virus can be successfully immunized with the same

dose when they are somewhat older, thus excluding the possibility that administration of an infectious agent immediately after birth may lead to tolerance and inability to grow protective antibodies later in life

Table 3 refers to infants fed the virus at the age of 10 days or less It shows that large doses of Type 1 virus can overcome the resistance of the intestinal tract to infection with polio virus in the newborn To investigate more thoroughly the relationship between apparent "immaturity" of the intestinal tract or definite physiological conditions prevailing in the intestinal tract in relation to age of the human infant, a study was undertaken in cooperation with Drs Cornely and Gyorgy on the effect of attenuated Type 1 virus on premature infants Table 4 summarizes the results of administration of Type 1 virus to nine premature infants on the third day of life Each infant received $10^{5 7}$ of tissue culture dose of the CHAT strain in a formula Although this dose is large, on occasion it failed to induce intestinal infection of term infants less than 30 days old In contrast, the virus multiplied in the intestinal tract of all premature infants without causing the slightest sign of illness or retardation of growth, which as indicated by gain in weight was progressing at a steady rate As shown in Table 5, the transplacental antibodies were present in the blood of four prematures who were submitted to serological investigation Although these results are not as yet complete, they indicate that during one month following vaccination the antibody titer was maintained In the absence of antigenic stimulus one would expect the titer to decrease if the antibody half-life figures obtained by Plotkin and others[3] in the case of term infants are applicable to the prematures

The results of these studies, communicated through the Pan American Health Organization to the kingdom of El Dorado resulted in a decision by the entire population that all newborns will be vaccinated with live oral vaccine Type 1 virus on the first day after birth, Type 3 upon a return visit one month after leaving the hospital, and Type 2 anytime thereafter This wise step taken by the population has resulted in the disappearance of polio virus from El Dorado and is in keeping with the wisdom which has brought the El Doradians to their present position of world leadership

Leaving the Southern Hemisphere and returning to the United States, one faces the problem of the behavior of attenuated polio virus in the most natural ambient of all, namely an average American family During the past year we have investigated the peregrinations of the three types of attenuated virus in 18 families living in Moorestown, New Jersey (the results of these studies are to be published) According to the sociological classifications of Stanley Warner, re

TABLE 4 PREMATURE INFANTS

SUCCESS AND SAFETY OF VACCINATION WITH CHAT, TYPE 1 ATTENUATED POLIOVIRUS VACCINE

INFANT No	BIRTH WEIGHT		DURATION OF EXCRETION IN DAYS AFTER VACCINATION*
	LB	GM	
1	2 5	1136	Day 3 to day 14+
2	4 2	1909	Day 3 to day 16+
3	2 7	1227	Day 3 to day 7+
4	4 3	1995	Day 7 to day 9+
5	4 6	2091	Day 5 to day 13+
6	2 5	1150	Day 2 to day 9+
7	3 4	1559	Day 2 to day 23+
8	4 9	2220	Day 2+
9	4 8	2160	Day 3 to day 13+

* All infants gained weight and thrived without signs of illness

TABLE 1 RELATIONSHIP OF AGE OF INFANT TO SUCCESS OF VACCINATION

Strain—	CHAT	Wistar	P-712	Fox	Total*
	Ratios of Successful to Total Vaccinations in Each Category				
Age (days)					
30	10/13	3/4	2/2	8/10	23/29
31–60	12/15	3/3	1/2	6/10	22/30
60	9/9	2/2	16/18	14/14	41/43

* $X^2 = 7.1$, $p < .05$, for data in "total" column

TABLE 2 RELATIONSHIP OF TRANSPLACENTAL ANTIBODY LEVEL TO SUCCESS OF VACCINATION

Strain—	CHAT	Wistar	P-712	Fox	Total*
	Ratio of Successful to Total Vaccinations in Each Category				
Pre-vaccination Reciprocal Titer					
256	6/8	3/4	1/1	5/8	15/21
8–255	13/15	4/4	4/5	10/13	31/37
<8	12/14	1/1	14/16	13/13	40/44

* $X^2 = 4.0$, $p < .10$, for data in "total" column

TABLE 3 RESPONSE OF INFANTS LESS THAN TEN DAYS OLD TO VACCINATION

Age in Days	Fecal Excretion		Pre-vaccination	Post-vaccination	
	Onset (Days)	Duration (Days)	Titer (Cord Blood)	Age in Days	Titer
0	2	10	16	113	32
5	14	14†	512	34	32
				147	512
7	3	25	256	170	128
8	9	32	4	37	256
9	3	15	128	126	2048

† = Virus excretion replaced after this number of days by feeding of another type of virus.

ceptible children, or 46%, excreted Type 1 virus, 6 of 21, or 29%, excreted Type 2 virus and 14 of 27, or 52%, excreted Type 3 virus The second part of this table under the heading of tertiary spread refers to those families where at least one child in the family had excreted virus as the result of contact infection Although the numbers are rather small, it is interesting to note that the percentage of susceptibles excreting virus by possible tertiary spread is approximately the same as for secondary spread

TABLE 6 VIRUS INFECTION RATIOS DURING INDEX CHILD FEEDINGS

	Polio Type I	Polio Type II	Polio Type III
Ind Child	18/18	18/18	18/18
Parents	4/36	1/36	0/36
Siblings	13/35	6/35	14/35

TABLE 7 PERCENTAGES OF SUSCEPTIBLES VACCINATED BY CONTACT

	Polio Types I		II		III	
2° *Spread*		%		%		%
Adults	4/15*	27	1/15	7	0/14	0
Children	12/26	46	6/21	29	14/27	52
"3° *Spread*"						
Adults	1/3	33	0/1	—	—	—
Children	3/5	60	1/6	16	5/9	56

* No infected over No susceptible

Illness in family contacts of index children is shown on Table 8 The minor illnesses included anorexia, dermatitis, chicken pox, erythema infectiosum There were no major illnesses observed during the entire study and there is no difference in the incidence of illnesses between those who excreted polio virus and those who did not In any given week there was no excess of illness in relation to virus feeding or contact infection, thus confirming the inability of attenuated virus in this particular study to cause disease

The antibody response in index children and their contacts to administration of live virus is shown in Table 9 In this case the data were obtained only in individuals who had no antibodies to the type to which they were exposed It will be observed that all index children develop antibodies against Types 1 and 3 Of ten susceptible adult contacts three developed antibodies against Type 1 but none of the 9 non-immune against Type 3 developed antibodies against Type 3. Data for contact siblings indicate a higher ratio of sera conversion—7 out of 11 in Type 1 and 4 out of 11 in Type 3

TABLE 8 MINOR ILLNESSES IN FAMILY CONTACTS OF INDEX CHILDREN

Polio Types	I	II	III
Adults	%	%	%
S Inf	2/4 (50)	0/1(-)	-/- (-)
S Uninf	2/11(18)	2/14(14)	5/14(36)
R Uninf	1/21(5)	2/21(10)	2/22(9)
Children			
S Inf	2/12(16)	2/6 (33)	2/14(14)
S Uninf	6/14(43)	1/15(7)	7/11(64)
R Uninf	2/9 (22)	3/14(21)	1/8 (13)

S=Susceptible R=Resistant

Pharyngeal excretion of the virus was noted in several of the index children However, no difference in spread was observed in the families of these children as compared to other families

What we will show now are samples of patterns of spread of the virus through several families involved in the study In Table 10 it will be seen that in this family the mother, father and one sibling started to excrete Type 1 virus within 12 to 37 days after the index child had been fed this same type of virus None of them had antibodies against Type 1 virus before contact infection and all developed such antibodies Two other siblings excreted Type 3 virus Excretion started on the 10th day after the index child was fed this strain Of these two, one had antibodies induced by inactivated virus vaccine, whereas one did not Both developed antibodies against this virus following contact infection

In Family B (Table 11) the 2½-month-old index child showed neutralizing antibodies (probably of transplacental origin) following vaccination with inactivated virus vaccine but nevertheless excreted all three types of virus when exposed His mother and father had antibodies against all these types and did not become infected by contact exposure The three siblings

TABLE 5 PREMATURE INFANTS
SUCCESS AND SAFETY OF VACCINATION WITH CHAT, TYPE 1 ATTENUATED POLIOVIRUS VACCINE
• ANTIBODIES •

INFANT No	BIRTH WEIGHT LB	BIRTH WEIGHT GM	DURATION OF EXCRETION	RECIPROCALS OF NEUTRALIZING ANTIBODY TITERS: PRE-VACCINATION	RECIPROCALS OF NEUTRALIZING ANTIBODY TITERS: ONE MONTH POST-VACCINATION
1	2 5	1136	12*	1 8* (1.8)†	1 8
2	4 2	1909	14*	1 16	1 32
3	2 7	1227	5*	1 256 (1 256)	1 64 (1·128)
4	4 3	1995	3*	(1 256)	(1 128)

* Metabolic inhibition test
† Gard test (in parentheses)

cently revised by Vance Packard, all these families belong either to the upper-middle or middle class Because of the better than average intelligence of the adult members of the families, it was possible to obtain good cooperation throughout the trial period There was one child in each family who had no prior contact with polio virus as determined by lack of antibodies in his serum and had no vaccination with inactivated virus prior to the beginning of the trial All of the other children and some of the adults had been inoculated some time before with three injections of inactivated virus vaccine by the family physician

At the start of the study all adult members of the families who had no prior immunization were given two inoculations of inactivated virus vaccine at three week intervals, the same schedule was applied to the non-immunized child, referred to hereafter as the index child The age of the index child varied between 2 and 18 months, whereas the upper limit of the siblings was 10 to 11 years It may be noted that 65% of the children who received three injections of inactivated virus vaccine developed antibodies against Types 1 and 3 and 100% developed antibodies against Type 2 The index child, in each case the youngest of the family, was fed the three attenuated types of polio virus in succession at three week intervals. The first administration consisted of the CHAT Type 1 strain followed three weeks later by Fox Type 3 and then three weeks after by the P712 strain of Type 2 virus During the ensuing 15 weeks a visiting nurse collected stool specimens from all family members at bi-weekly intervals. In addition, pharyngeal swabs were obtained from index children Close supervision by the nurse and family physician was maintained during the entire study for any signs or symptoms of illness

At the end of this observation period all members of the family were fed the three attenuated types in succession At the same time the index child was refed Stool specimens were again collected following feeding of each virus Blood specimens were again collected before and several times after the study had begun All 18 index children excreted in their stools the type of virus fed to them Table 6 shows the extent of the spread of the three types of virus for parents and for siblings and, as was expected, the incidence of contact infection among parents was much lower than that among siblings I should add that in Type 1 infection, four of 36 parents excreted the virus. In Table 7, the same figures are shown under the second spread for susceptibles among parents and contact children In this case the term *susceptible* is *applied to* those subjects whose sera had no antibodies to the type of virus given at the beginning of the study or to individuals who had antibodies after inoculation with the inactivated virus vaccine but nevertheless were excreting the attenuated virus after contact with the index child It may be observed that in this case four of 15 susceptible adults (27%) excreted Type 1 virus, 1 of 15 excreted only Type 2, and none of the 14 excreted Type 3 virus In contrast, 12 of 26 sus-

TABLE 11. VACCINATION HISTORY OF FAMILY B

SUBJECT	AGE	VIRUS EXCRETION DAYS AFTER I C FED TYPES			NEUTRALIZING POST-SALK PRE-LIVE VIRUS			ANTIBODIES POST-LIVE VIRUS TO INDEX CHILD		
		I	II	III	I	II	III	I	II	III
I C	2 5 m	2	3	2	(+)	(+)*	0	+	+	+
F	27 y	–	–	–	+	+	+	+	+	+
M	29	–	–	–	+	+	+	+	+	+
S	6	8	–	–	+	+	+	+	+	+
S	4 5	9	–	–	+	+	+	+	+	+
S	2	10	–	16	±	+	+	+	+	+

* Means transplacental antibodies

TABLE 12 SPREAD OF TYPE III VIRUS IN FAMILY I

		WEEKS AFTER FIRST FEEDING						
		4	5	6	7	8	9	10
I-1	0 5 y	III		II				
I-2	45							
I-3	41							
I-4	11			III				
I 5	10			III				
I-6	7				III			
I-7	2		III					

siblings, all of whom showed the presence of antibodies before contact with the exposed child The two younger siblings of the family whose serum also showed the presence of antibodies after administration of inactivated virus vaccine and who had not acquired Type 1 infection as a result of contact were found to excrete Type 1 virus upon exposure

The pattern of virus excretion upon re exposure in the case of Types 3 and 2 is shown in Table 14 The parents of the index child who had no antibodies against Type 3 virus and who were infected by contact infection became intestinal carriers upon re exposure to Type 3 virus However of greater interest perhaps is the fact that all four siblings who became infected by contact infection with Type 3 virus and who excreted the virus for a considerable length of time became, with one exception, intestinal carriers upon re exposure This occurred in the presence of homotypic neutralizing antibodies and although the duration of intestinal carriage was much shorter than after contact infection, in the case of one child it exceeded 21 days One of the siblings who had antibodies following vaccination with inactivated virus vaccine became infected with Type 2 virus upon direct administration

In family J parents and siblings had not suffered from previous contact infection and direct exposure to the CHAT virus resulted in intestinal infection Direct feeding of Fox virus to the same family resulted in an infection in the mother, who had no previous contact infection and had no antibodies against Type 3 virus An interesting observation relates to the same person in the case of P712 Type 2 virus where in spite of a contact infection, she again became infected upon direct feeding of Type 2 virus

In the case of Family H (Table 15) no contact infection occurred with Type 1 virus and all but one member of the family excreted this type of virus upon direct feeding The data on Type 3 virus excretion are shown in the next column Three of the five contacts of the index child who had no previous contact infection with the Fox strain excreted virus upon direct feeding Of these three, two had antibodies against Type 3 virus In the case of Type 2 virus two siblings who became infected through contact resisted the virus upon direct feeding One of the siblings who had no contact infection became infected

TABLE 9 ANTIBODY RESPONSE IN SERONEGATIVE INDIVIDUALS TO LIVE VIRUS VACCINATION OF FAMILIES

VACCINATION OF	ANTIBODIES TO TYPES					
	I		II		III	
	PRE-VACC	POST-VACC	PRE-VACC	POST-VACC	PRE-VACC	POST-VACC
Index Child						
Index children	0/18	18/18	0/18	17/18	0/18	18/18
Adult contacts	0/10	3/10	0/1	0/1	0/9	0/9
Siblings	0/11	7/11	—	—	0/11	4/11
Family						
Adults	0/7	4/7	0/1	1/1	0/9	8/9
Children	0/4	3/4	—	—	0/7	6/7

TABLE 10 VACCINATION HISTORY OF FAMILY Q

SUBJECT	AGE	VIRUS EXCRETION DAYS AFTER I C FED TYPES			NEUTRALIZING POST-SALK PRE-LIVE VIRUS			ANTIBODIES POST-LIVE VIRUS TO INDEX CHILD		
		I	II	III	I	II	III	I	II	III
I C	2 5 m	3	1	3	0	(+)*	0	+	0	+
F	27 y	37	—	—	0	0	+	+	0	+
M	28	13	—	—	0	+	+	+	+	+
S	3	—	—	10	+	+	+	+	+	+
S	1	12	—	10	0	+	0	+	+	+

* Means transplacental antibodies

who had antibodies following vaccination with inactivated virus vaccine excreted Type 1 virus within 8 to 10 days after the index child was fed One of the siblings also became infected with Type 3 virus upon contact exposure

Spread of Type 3 virus through four siblings of Family I is shown in Table 12 It will be observed that all these began to excrete virus within three weeks after the index child was fed Type 3 virus Parents of the child did not become infected

As has been mentioned before, at the end of the initial 15-week observation period, and after completion of the study on primary exposure of the index child and contact infection of family members, all members of all families were fed the types of virus The results are shown in Table 13 The index child who had a trace of antibody as a result of immunization with inactivated virus vaccine was resistant to refeeding with Type 1 virus The same resistance was observed in the case of his parents and two

TABLE 15 PATTERNS OF VIRUS EXCRETION IN PERSONS RE-EXPOSED TO ATTENUATED POLIOVIRUS FREQUENT BUT VARIABLE RESISTANCE TO RE-INFECTION IS DEMONSTRATED

Family Member†	CHAT Excretion After Contact	CHAT Excretion After Direct Feeding	FOX Excretion After Contact	FOX Excretion After Direct Feeding	P-712 Excretion After Contact	P-712 Excretion After Direct Feeding
H 2*	No	Yes 18	No	Yes 14	No	No
3*	↓	No	↓	No	↓	No
4	↓	Yes 18	↓	No	Yes 16	No
5	↓	Yes 10	↓	Yes 17	No	Yes 17
6	↓	Yes 13	↓	Yes 42	Yes 41	No
N 2*	No	No	No	No	No	Yes 10
3*	↓	No‡	↓	No	↓	No
4	Yes 63	Yes 8	↓	Yes 27†	↓	?

† Index child not included ‡ Yes 21 X on re feeding CHAT
* Adult

TABLE 16 REINFECTION RATIOS FOR TYPE I POLIO VIRUS (CHAT)

		%		
Vaccinated				
Index Children	3/17	18	*7/33	21%
Contact Adults	1/4	25		
Contact Siblings	3/12	25		
Non-Vaccinated				
Natural Immunes	0/7	—	9/12	75%
Salk-Imm Adults	5/8	63		
Salk-Imm Siblings	4/4	100		

* All but one of seven had sporadic excretion

still available today for a study of the levels of the Type 2 neutralizing antibodies[3] The results of neutralization tests, summarized in Table 17, indicate that seven of the eight subjects had Type 2 antibody titers, 8 to 9 years after ingestion of virus, ranging from 1 16 to 1·256, with a median of 1 84 Although, in comparison with Type 2 antibody levels shown 1-2 months after virus administration, the current titers are slightly lower, it should be remembered that some of the original titers were measured by mouse neutralization (before tissue culture techniques had been developed) and may not be directly comparable

Six subjects who had been fed attenuated Type 1 virus in 1953-54 were also now available for determination of Type 1 antibody levels in their sera (Table 18) One of these subjects had also been fed the TN strain and had acquired antibodies to both types The antibody levels of the remaining subjects, 4½ years after virus feeding, were on the average equal to those obtained one to three months after the feeding

It was possible, some workers contended, that subsequent natural infection could act as a booster and help maintain the level of antibodies against the type originally administered In order to determine the likelihood of natural homotypic infection in the course of the years after actual vaccination, sera obtained recently from subjects in both trial groups were tested for heterotypic antibody titers The results presented on Table 19 indicate that heterotypic antibody was absent in the majority of tests. Although the groups under study were small only about 1/3 of the sera tested had heterotypic antibody If we are permitted to judge by comparison, the probability of a homotypic booster infection may be of the same order

In the same type of study thirteen infants who received attenuated strains, representing all three types of virus at 5 days to 5 months of age were bled 11-28 months later and their sera studied for homotypic antibody In most cases, the immuno inhibition test developed by Gard was

TABLE 13. FAMILY I

	Exc I	Exc II	Exc III	Antibodies Before I	Before II	Before III	After I	After II	After III	Exc Fam I
IC	2	2	6	(±)	(±)	(±)	+	+	+	−
F				+	(+)	(+)	+	(+)	0	−
M				+	+	0	+	+	0	−
C1			23	+	+	(+)	+	+	+	−
C2			23	+	+	(+)	+	+	+	−
C3			27	(+)	+	(+)	(+)	+	+	+
C4			16	(+)	+	0	(+)	+	+	+

TABLE 14 PATTERNS OF VIRUS EXCRETION IN PERSONS RE-EXPOSED TO ATTENUATED POLIOVIRUS FREQUENT BUT VARIABLE RESISTANCE TO RE-INFECTION IS DEMONSTRATED

Family Member†	Chat: Excretion After Contact	Chat: Excretion After Direct Feeding	Fox: Excretion After Contact	Fox: Excretion After Direct Feeding	P-712: Excretion After Contact	P-712: Excretion After Direct Feeding
I 2*	No	No	No	Yes 3	No	No
3*	|	No	↓	Yes 17	|	No
4	|	No	Yes 37	Yes 14	|	Yes 17
5	|	No	Yes 30	Yes 10	|	No
6	|	Yes 12	Yes 56	Yes 21†	|	?
7	↓	Yes 8	Yes 40	No	↓	No
J 2*	No	Yes 42	No	No	Yes 5	No
3*	|	Yes 10	↓	Yes 21†	Yes 5	Yes 10
4	↓	Yes 27	Yes 31	No	No	?

† Index child not included
* Adult

upon direct feeding Both parents resisted infection.

Table 16 gives the summary of the results in relation to Type 1 virus Of 8 adults immunized with inactivated virus vaccine, five became infected by direct feeding Four out of four children also became infected None of the 7 subjects who were found to be naturally immune were infected. The re infectivity ratio for vaccinated and infected by contact subjects was 21%. Of the 7 persons, 6 developed sporadic excretion of the virus. The studies along the same line with Type 2 and 3 virus are not as yet completely evaluated. It seems, however, that the results with the Type 3 virus will differ in this respect. The infectivity ratio of children who previously excreted the same type of virus as a result of contact infection will be somewhat higher.

The first child ever to receive live virus orally for immunization purposes was fed TN Type 2 strain of poliovirus by Dr. Jervis, Mr Norton and Koprowski in February 1950[2] During the ensuing year the same strain was administered orally to 20 more children who had no antibodies to Type 2 virus. Eight of these children were

TABLE 15 PATTERNS OF VIRUS EXCRETION IN PERSONS RE EXPOSED TO ATTENUATED POLIOVIRUS FREQUENT BUT VARIABLE RESISTANCE TO RE-INFECTION IS DEMONSTRATED

FAMILY MEMBER†	CHAT		FOX		P-712	
	EXCRETION AFTER		EXCRETION AFTER		EXCRETION AFTER	
	CONTACT	DIRECT FEEDING	CONTACT	DIRECT FEEDING	CONTACT	DIRECT FEEDING
H 2*	No	Yes 18	No	Yes 14	No	No
3*	|	No	|	No	↓	No
4	|	Yes 18	|	No	Yes 16	No
5	|	Yes 10	|	Yes 17	No	Yes 17
6	↓	Yes 13	↓	Yes 42	Yes 41	No
N 2*	No	No	No	No	No	Yes 10
3*	↓	No‡	|	No	|	No
4	Yes 63	Yes 8	↓	Yes 27†	↓	?

† Index child not included ‡ Yes 21 N on re feeding CHAT
* Adult

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Non-Vaccinated				
Natural Immunes	0/7	—	9/12	75%
Salk-Imm Adults	5/8	63		
Salk-Imm Siblings	4/4	100		

* All but one of seven had sporadic excretion

still available today for a study of the levels of the Type 2 neutralizing antibodies[3] The results of neutralization tests, summarized in Table 17, indicate that seven of the eight subjects had Type 2 antibody titers, 8 to 9 years after ingestion of virus, ranging from 1:16 to 1:256, with a median of 1 84 Although, in comparison with Type 2 antibody levels shown 1-2 months after virus administration, the current titers are slightly lower, it should be remembered that some of the original titers were measured by mouse neutralization (before tissue culture techniques had been developed) and may not be directly comparable

Six subjects who had been fed attenuated Type 1 virus in 1953 54 were also now available for determination of Type 1 antibody levels in their sera (Table 18). One of these subjects had also been fed the TN strain and had acquired antibodies to both types The antibody levels of the remaining subjects, 4½ years after virus feeding, were on the average equal to those obtained one to three months after the feeding

It was possible, some workers contended, that subsequent natural infection could act as a booster and help maintain the level of antibodies against the type originally administered In order to determine the likelihood of natural homotypic infection in the course of the years after actual vaccination, sera obtained recently from subjects in both trial groups were tested for heterotypic antibody titers The results presented on Table 19 indicate that heterotypic antibody was absent in the majority of tests Although the groups under study were small only about 1/3 of the sera tested had heterotypic antibody If we are permitted to judge by comparison, the probability of a homotypic booster infection may be of the same order.

In the same type of study thirteen infants who received attenuated strains, representing all three types of virus at 5 days to 5 months of age were bled 11-28 months later and their sera studied for homotypic antibody. In most cases, the immuno inhibition test developed by Gard was

TABLE 17 PERSISTENCE OF ANTIBODIES TO TYPE 2 POLIOVIRUS IN CHILDREN FED TN STRAINS (GROUP A)

Virus Dose Fed ($Log_{10}MPD_{50}$)	Virus in Feces (Days After Feeding)	Reciprocal of Neutralizing Antibody Titer (At Various Months After Feeding)			
		1–2 Months		56–101 Months	
		1/Titer	(Months)	1/Titer	(Months)
5.0	4, 9	600	(1)*	128	(101)
4.0	None	96	(1)	32	(100)
2.1	13	256	(1)	64	(94)
2.1	9, 13	256	(2)	64	(91)
2.1	9	422	(1)*	128	(94)
2.1	None	32	(1)*	16	(94)
2.1	None	378	(1)*	16	(94)
5.5	11, 17	128	(2)	256	(56)

* Mouse protective test

TABLE 18 PERSISTENCE OF ANTIBODIES TO TYPE 1 POLIOVIRUS IN CHILDREN FED SM STRAINS (GROUP B)

Virus Dose Fed ($Log_{10}TCD_{50}$)	Virus in Feces (Days Inclusive After Feeding)	Reciprocal of Neutralizing Antibody Titer (At Various Months After Feeding)			
		1–3 Months		47–56 Months	
		1/Titer	(Months)	1/Titer	(Months)
3.8	3–11	384	(2)	128	(56)
5.2	5–46	64	(3)	256	(52)
4.2	3–51	384	(1)	32	(50)
4.9	4–50*	96	(1)	16	(50)
4.0	4–34*	128	(1)	256	(48)
4.7	2–30	256	(1)	512	(48)
4.4	3–198*	32	(1)	64	(47)

* Excretion not studied beyond this time

TABLE 19 PRESENCE OF HETEROTYPIC POLIOVIRUS ANTIBODIES IN SERA (1958) OF SUBJECTS PREVIOUSLY GIVEN ATTENUATED VIRUS *

Subject	Group A Type 1	2	3	Group B Subject	Type 1	2	3
2	0	V	0	SC	V	V	+
5	+	V	+	JC	V	0	0
7	0	V	0	Bo	V	0	+
8	0	V	0	T	V	+	0
9	N	V	0	W	V	N	0
13	+(tr)	V	0	Ge	V	0	0
14	N	V	+	Go	V	+	0
Totals							
V		7				8	
N		2				1	
+		4				4	
0		8				8	

* Symbols 0=antibody titer—1 4, +=antibody titer—1 4, N=natural antibodies before feeding, V=homotypic antibodies to attenuated virus

used From results presented in Table 20 it will be seen that transplacental antibody was present *almost in all sera prior to virus feeding but that* it did not inhibit the active response to multiplication of poliovirus in the alimentary tract Except for one infant fed Type 2 virus, all the subjects maintained high levels of homotypic antibody 20-28 months after administration of virus

Similar results were obtained in the other group of infants as shown in Table 21, with the exception of one infant who received Type 3 virus at the age of 2½ months This infant was refed the same preparation of Type 3 virus 19 months after the original feeding, at which time *his homotypic antibody was O In spite of this* fact the results of tests on isolation of fecal virus after the second feeding indicated a resistance of the intestinal tract to reinfection with polio virus

The results of tests for heterotypic antibody in the same group of infants are shown in Table 22 The results in most cases are so unequivocally negative that there is no doubt that the virus fed originally, and not the booster infection, has except in a very small proportion of cases, maintained the homotypic antibodies for two years or more

The mechanism of antibody formation against a virus has not yet been fully elucidated, nor is it clear why antibodies persist indefinitely or almost so, when induced by exposure to live virus, in contrast to dead virus The facts, however lend support to the theory that the persistence of antibodies following "natural" exposure for approximately 35 years or longer, as observed by Paul and his associates,[4] and the duration of immunity following exposure to a living attenuated virus, may be equally long

I was rather uncertain as to how to end my presentation, and I have decided to cite two authors: one is Igor Stravinsky, who, in the book by Craft on conversations with Stravinsky, has this to say on advice to composers

> "I suppose psychology has studied the effects of various types of challenges on various groups and I suppose it knows what are normal responses and when they occur—in this case, when one begins to seek defense from new ideas and to rationalize them away"

And then further on he says

> "The very people who have done the breaking through are themselves often the first to try to put a scab on their achievement What fear tells them to cry halt? What security do they seek, and how can it be secure if it is limited? How can they forget that they once fought against what they have become?"

The next quotation, I am ashamed to say, is from my own paper, written in the beginning of 1955 for a conference on poliomyelitis at the New York Academy of Sciences I am less bothered now quoting it because, to many of you, it will sound as Dr Dick would write it although at that time he had not as yet given his attention *to the field of poliomyelitis* Well, here it is

> 'Now, a few words about future steps in live virus immunization Most of the work so far, was accomplished in homes for the mentally defective Other investigators are performing similar work among volunteers in prisons If the research work continues at the present pace and remains limited to these

TABLE 20. HOMOTYPIC ANTIBODIES TO POLIOVIRUS IN INFANTS FED ATTENUATED STRAINS OF VIRUS

VIRUS TYPE FED	AGE AT VACCINATION (MONTHS)	VIRUS DOSE FED (LOG_{10} TCD_{50})	RECIPROCAL OF NEUTRALIZING ANTIBODY TITER FOR ANTIBODIES TO TYPE FED (AT VARIOUS MONTHS AFTER FEEDING)			
			0	1 MONTH	11–27 MONTHS	
1	3	4 7	64	64	512	(28)
2	5	5 3	24	1024	6	(25)
1	5	5 1	6	179	1024	(25)
1	3	2 8	16	256	256	(24)
1	2	4 1	48	256	1024	(23)
1	6	4 8	<4	362	1024	(20)
1	5 days	4 8	256	64	512	(20)

TABLE 21 HOMOTYPIC ANTIBODIES TO POLIOVIRUS IN INFANTS FED ATTENUATED STRAINS OF VIRUS

VIRUS TYPE FED	AGE AT VACCINATION (MONTHS)	VIRUS DOSE FED (LOG_{10} TCD_{50})	RECIPROCAL OF NEUTRALIZING ANTIBODY TITER FOR ANTIBODIES TO TYPE FED (AT VARIOUS MONTHS AFTER FEEDING)			
			0	1 MONTH	11–27 MONTHS	
1	2	5 8	96	64	712	(15)
1	3	7 5	24	256	+	(12)
1	1	7 5	48	64	128	(11)
2	5	5 3	64	256	>1024	(25)
2	3		64	256	256	(21)
3	2½	5 5	64	64	0	(14)
3	2	5 5	256	64	>1024	(14)

population groups, then—giving a hint to Dr Wilson—by 1984 the inmates of asylums and of prisons in the United States will become the only two groups of society *permanently* immunized against poliomyelitis. Paradoxically speaking, a man who may have had the misfortune to be sentenced for life will be given the great chance of being protected forever against poliomyelitis"

And further on I said:

"If certain scientists are still haunted by the specter of live virus vaccines, they had better adopt, toward this new era of immunization, an attitude similar to that of Horatio announcing the appearance *of Hamlet's father's ghost* 'Look, my Lord! It comes'."

I regret to state that, although this is 1959 and not 1984, the statement concerning "in-

TABLE 22 PRESENCE OF HETEROTYPIC POLIOVIRUS ANTIBODIES IN SERA (1958) OF SUBJECTS PREVIOUSLY GIVEN ATTENUATED VIRUS*

GROUP C

SUBJECT	TYPE 1	TYPE 2	TYPE 3
C 2	V	V	O
C 7	V	+	O
C10	V	O	O
C11	V	O	O
C14	V	O	O
C26	V	O	O
C32	V	+	+
C40	V	O	O
C46	V	O	O
C 6	+	V	O
C20	O	V	O
C34	+	O	V
C37	O	+	V

TOTALS	
V	14
N	–
+	6
O	19

* Symbols O=antibody titer 1 4, +=antibody titer—1 4 N=natural antibodies before feeding V=homotypic antibodies to attenuated virus

stitutional" immunization holds true for the northern part of the Western Hemisphere, Great Britain, and a few other countries. For them this is still "a ghost"; for other countries it has "materialized" This leaves us only with hope, and "of all the plums of the wedding cake, hope is the sweetest."

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TABLE 20. HOMOTYPIC ANTIBODIES TO POLIOVIRUS IN INFANTS FED ATTENUATED STRAINS OF VIRUS

Virus Type Fed	Age at Vaccination (Months)	Virus Dose Fed (Log_{10} TCD_{50})	Reciprocal of Neutralizing Antibody Titer for Antibodies to Type Fed (At Various Months After Feeding)			
			0	1 Month	11-27 Months	
1	3	4 7	64	64	512	(28)
2	5	5 3	24	1024	6	(25)
1	5	5 1	6	179	1024	(25)
1	3	2 8	16	256	256	(24)
1	2	4 1	48	256	1024	(23)
1	6	4 8	<4	362	1024	(20)
1	5 days	4 8	256	64	512	(20)

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Virus Type Fed	Age at Vaccination (Months)	Virus Dose Fed (Log_{10} TCD_{50})	Reciprocal of Neutralizing Antibody Titer for Antibodies to Type Fed (At Various Months After Feeding)			
			0	1 Month	11-27 Months	
1	2	5 8	96	64	712	(15)
1	3	7 5	24	256	+	(12)
1	1	7 5	48	64	128	(11)
2	5	5 3	64	256	>1024	(25)
2	3		64	256	256	(21)
3	2½	5 5	64	64	0	(14)
3	2	5 5	256	64	>1024	(14)

population groups, then—giving a hint to Dr Wilson—by 1984 the inmates of asylums and of prisons in the United States will become the only two groups of society *permanently* immunized against poliomyelitis Paradoxically speaking, a man who *may have had the* misfortune to be sentenced for life will be given the great chance of being protected forever against poliomyelitis."

And further on I said:

"If certain scientists are still haunted by the specter of live virus vaccines, they had better adopt, toward this new era of immunization an attitude similar to that of Horatio announcing *the appearance of Hamlet's father's ghost* 'Look, my Lord! It comes'."

I regret to state that, although this is 1959 and not 1984, the statement concerning "in

But I do not think we are mislead by the inner meaning.

I also have a question based on these studies, which are not as easy to grasp at first glance as they are for a person who has spent a lot of time putting the data together; what would be your impression now of the optimum time after birth for the administration of attenuated poliovirus?

DR. KOPROWSKI This is not an easy question to answer An infant, within hours after birth, can be fed one million or more tissue culture doses of virus and become immunized If smaller amounts of virus are to be used, then the optimum age for vaccination would be two months or older The prematures seemed to be as responsive to oral vaccination as term infants 60 days old or older. This has to be confirmed when a large number of prematures will be fed live attenuated virus

DR SABIN. I just want to say that I was the one who asked the question, and I would like to propose that perhaps further study of this question is indicated

Our pediatrician friends, who spent many years studying the peculiarities of the intestinal tract of premature and newborn infants—perhaps there might be comments on this—are impressed with the acidity that may be prevalent during the first few months after birth as a result of the intestinal flora

Many of these strains have been shown to be capable of lesser absorption and also, perhaps, lesser multiplication and spread from one to the other

This may be one of the factors in Dr Koprowski's observations, I wonder if Dr Wegman—from his extensive knowledge of the intestinal tract of newborns—may have some comment

DR WEGMAN Dr Sabin's question is rather difficult to answer, but I do feel certain that the amount of individual variation in premature infants is probably so great that I would be very hesitant to assume that any theoretical barriers existed because of variation in the flora due to age of the baby Acidity depends to some extent on what the baby is fed, and also upon the environment and the organisms with which the baby may be infected in the ordinary course of events as occurs very promptly after birth

DR BELL: The data presented by Dr Koprowski on the natural spread of attenuated virus invites comparison with the natural spread of wild poliovirus In our study of the Junior Village nursery group we observed 193 natural poliovirus infections. Children were generally tested for virus daily for the first 10 days after admission and once a week thereafter and at onset of illness. We found that the rate of spread to newly admitted children depended upon the number of children in the nursery environment who were shedding virus When four or more of the 60 odd institutionalized children were shedding virus, it generally took 8, 10, or 12 days before newly admitted children were infected

Dr. Fox, I believe, and others also, have shown that the spread of natural infections in a family proceeds rather rapidly One would think that the spread in families would be as rapid, if not more rapid, than in the institutionalized group of children

Now, Dr Koprowski shows that with artificial feeding of attenuated virus, there was quite a slow rate of spread in families, 10, 13, 17, 37 days I am wondering if this apparent delay suggests that the attenuated virus may have to revert to normal virus before it spreads

DR FOX I have a question but before going into it I would like to comment on Dr Bell's remarks

Our data, from the standpoint of natural infection, do not lend themselves to too much interpretation as to rapidity of spread because we very rarely were able to know exactly when a virus was introduced into a particular household We will have some data to be presented shortly, where we do know when virus was introduced into a household and I think it will show some fairly rapid spread

My question stems perhaps from the fact that I failed to get one of the essential facts that Dr Koprowski was presenting He made a point of the difference between the premature infant, which was fed within the first two or three days of life and the infants who were full-term but who were fed over periods ranging up to and presumably beyond sixty days of life.

I am wondering how many of these full-term infants were actually fed in the first two or three

DISCUSSION

DR. MELNICK In his remarks at this Conference, Dr. Koprowski has indicated that there should be *a moratorium on the use of monkeys* in studying the virus excreted by children fed the vaccine strains, and in this paper just presented, no monkey work has been included I do not believe that this omission is justified

It seems that safety in Dr Koprowski's trials is being measured directly in children. However, if small numbers are used, we have little indication of the safety of feeding any poliovirus, in view of the low paralytic rate in those infected with virulent strains I would like to ask Dr Koprowski how many cases he would have expected if his group had been fed virulent poliovirus

Data are available on the infection of children with virulent Mahoney virus when it slipped through the inactivation process at the time of the Cutter incident From the data on the injected children and on their familial contacts, and from data obtained during epidemic periods, exposure to virulent strains may result in only a single paralytic case for each hundred or so who become infected. Thus in any field trial with attenuated strains there must be well over 100 triple negatives in any group tested before it can be said that the vaccine has been proved safe

Dr Koprowski has spoken chiefly of the data obtained in El Dorado, for it must be admitted that the few susceptible children fed in the United States contribute little to the question of safety of the vaccine in man. Would he tell us how many triple negative children in El Dorado participated in the field trials, and how many were already infected with enteroviruses which interfere with the successful implantation of poliovirus vaccine in the alimentary tract? Such data are essential in interpreting the results of the El Dorado trials

DR KOPROWSKI It is sometimes customary for discussants to put words into the mouths of speakers, words which were never said. Doctor Melnick's accomplishments are even more subtle He has put thoughts into my head and attempted to present you with these as my own thoughts Please note that it is he, not I, who thinks that *I was proud of the fact of* not using monkeys, but using man directly, in view of the fact that "safety in Dr. Koprowski's trials is being measured directly in children"

Dr. Melnick knows very well that strains of virus used in this trial have undergone many "monkey safety tests," and that these virus preparations were fed to many thousands of children and adults before the present trial was undertaken.

There is no sense in interjecting emotional problems into scientific discussions. The study I have presented today consisted of three parts The first dealt with susceptibility of the intestinal tract of a newborn or a premature to infection with attenuated strains of poliovirus, and the influence of transplacental antibody on the course of such an infection In the second part, we studied the inter-familial spread of the attenuated virus under "normal" living conditions Here we studied an evaluated occurrence of minor and major illnesses in the vaccinees and in their contacts The third part dealt with the duration of immunity following administration of live attenuated strains This was not a safety trial except for the fact that no cases of illness attributed to the vaccine were observed

DR. MELNICK Then Dr Koprowski should change the heading of one slide, at least—the one which is labelled "susceptibility and safety" of the vaccine to infants

DR KOPROWSKI We are discussing now, not science but semantics. The attenuated strains were "safe" for infants who received them because none of them showed signs of illness I am quite happy with the heading of the slide and I do not intend to change it.

DR SABIN I think Dr Koprowski's report has rendered us a very important study There is an absolute necessity of understanding the behavior of attenuated viruses in El Dorado, or anywhere else Some people speak colorfully, he does

But I do not think we are mislead by the inner meaning

I also have a question based on these studies, which are not as easy to grasp at first glance as they are for a person who has spent a lot of time putting the data together, what would be your impression now of the optimum time after birth for the administration of attenuated poliovirus?

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I am wondering how many of these full-term infants were actually fed in the first two or three

days of life, because it seems to me that it may not be a difference between prematurity and full-term, but how long the individual actually has been exposed to the postnatal environment

DR KOPROWSKI: One more participant wants to ask a question Following that I shall attempt to reply to all questions Perhaps Dr. Plotkin would like to comment on Dr. Fox's question

DR PLOTKIN We fed three infants within the first three days of life. Included was one infant fed within 24 hours of birth, in whom we obtained a successful vaccination accompanied by excretion and an antibody rise Two of the infants in the less than 10-day-old group were not successfully vaccinated I do not think we have enough infants in this very young group to make any comparison to infants 10 days to 2 months old

I would like to make two comments, the first concerning Dr Bell's question in some of those families we consider that there is evidence for spread of virus from a secondary contact that is, from a contact of the vaccinee to another sibling in the family—in other words tertiary spread of virus The Type 3 infection shown by Dr Koprowski, occurring for 37 days in child I-4, for example, circumstantially appears to have been spread from one sibling to another The index child had ceased to excrete the Type 3 virus when this infection occurred In other families there are also examples of possible tertiary spread

The second comment is with reference to the point made by Dr Melnick His comment is an argument often used in criticism of safety testing of live virus vaccine, and to be sure there is no question that there are viruses circulating in nature which cause very few symptomatic poliomyelitis infections: perhaps one in a thousand, or fewer.

But, on the other hand, when one asks what would happen if one fed Mahoney virus, a truly virulent virus, I do not feel it is fair to use the Cutter incident as an indication For example, Craig and Brown's recently published studies have shown that, using the dosage which is employed in the live virus vaccination, the Mahoney virus can be highly virulent for monkeys and paralyze 70 or 80 per cent of them, as I recall Secondly, the Mahoney virus in the Cutter vaccine was in a sense attenuated or at least treated with formaldehyde The dosage in the vaccine, therefore, may not have been very high I wonder if Dr. Bodian would care to comment on what he thinks would happen if the Mahoney virus in dosages of 100,000 or two million particles were to be fed to chimpanzees

CHAIRMAN STUART-HARRIS: I am very glad that Dr. Koprowski mentioned the fact that his early studies were being made and were done in homes for children, because this has a very great bearing on the interpretation of the antibody studies.

DR. DICK I realize that this does not apply to modern strains, but we know that TN virus can revert to a highly paralytic strain In the community of children in a children's home, the immunity which Dr Koprowski is now measuring and claims to be durable is probably the result of reinfection with virulent reverted virus

In our studies with TN virus, the levels of antibodies we found in children one year after vaccination were of the same order as those reported in non-immune children who had received two injections of formalized vaccine the year before A number of TN-vaccinated children were found to have had a marked decline in their neutralizing antibody level over the year

Our previous findings that 23 per cent of the children failed to develop antibody after vaccination were confirmed As far as SM is concerned, we found a decline in antibody in 6 out of 7 individuals we examined

I realize that this does not necessarily relate to strains which are being used at present—the currently acceptable strains—but we must not draw any conclusions on the antibodies we may expect or the duration of antibodies we may expect from studies with TN vaccine which is now, as far as most people are concerned, not acceptable Studies which were made in institutes, or in children's homes where reinfections of the children with reverted virus can occur, may show a durable immunity With TN virus it may take quite a while before the virus reverts to a virulent form The immunity measured under these conditions may be durable and similar to what might be obtained with a virulent Type 2 virus

DR BODIAN A simple technical question for

clarification. In the tables in which Dr Koprowski showed a comparison of antibody titers, were the sera run simultaneously?

CHAIRMAN STUART HARRIS: If you do not mind, Dr Koprowski will answer these questions at the end of the discussion.

DR HAMMON: I would like to mention some studies that were carried out with apparently quite avirulent strains naturally infecting American military families living on an Air Force base in the Philippines. This was a longitudinal-type study similar to the one that Dr Fox carried out. In this instance we took swabs and blood specimens at frequent intervals over a period of four months, in these normal families. During this period of time poliovirus Types 1, 2, and 3 invaded this community, went through many of these families, not producing a single case of recognizable illness due to poliomyelitis infection.

So here we have relatively avirulent wild strains passing through families.

I would like to point out the remarkable similarity of the behavior of these viruses and the one that Dr Koprowski has talked about and others. They behaved somewhat differently from the studies which we and others have carried out in families where a case of paralytic poliomyelitis occurred. There, quite frequently nearly 100 per cent of the susceptibles become infected.

However, in these families in the Philippines where these avirulent strains were present, it was *not* nearly 100 per cent of any of the families that were affected with the virus. The susceptibles among the parents frequently were not infected and occasionally they were. A fairly high proportion of the siblings became infected but by no means all of them.

With respect to antibody response, these persons developed antibody as a result of infection, excreted virus, but *over a period of three or four months* when we observed them, a good many of these had rather marked antibody titer falls. By no means all fell, for many of them maintained a constant antibody, but some of them, by the CP type of test, fell off from antibody titers of a thousand or greater to titers of 1 to 8 or 1 to 16 at the end of three or four months.

So natural infection does not necessarily maintain antibodies at a high level.

Other studies which we made following up gamma globulin evaluation programs in Sioux City, Iowa, and in Texas, where we bled children several months after the original infection was discovered, showed that actually *one or two* children that had very high titers to the virus which infected them had lost all detectable antibody at eight-month intervals. Approximately 15 to 20 per cent had dropped to very low levels where they could merely be detected.

With respect to the Philippine studies on the rapidity of progression of the virus through the family, we were able to determine this in many instances because of the frequent collection of specimens.

The intervals that Dr Koprowski showed were, I would say, entirely comparable to the intervals which we found within these families.

DR KOPROWSKI: Dr Bell raised the question that an attenuated virus *may* have to revert to normal virus (although I think that Dr Bell would have had difficulty defining what a "normal" virus is) before it spread. I showed you yesterday a study of genetic markers of the CHAT and Fox strains which were employed in the Moorestown study. Virus isolated from the father of one of the vaccinees representing the third passage of the CHAT strain through human intestinal tract had the same properties as the virus *originally* fed. This "third passage" strain failed to induce signs of paralysis or lesions in monkeys injected intracerebrally with 10^7 tissue culture doses. I do not know whether this is a 'normal' or 'abnormal' strain, but I *do* know that it retained several characteristics of the attenuated virus used for immunization purposes.

If I may comment further along these lines, it seems to me that the speed of spread of poliovirus is not related to the "normalcy" of the virus but to *its infectivity for man*. We know that the CHAT virus is of relatively low infectivity for man as compared with other attenuated strains such as, for instance, the SM strain used some time ago. I doubt if Dr Bell has studied the infectivity end point for man of polio strains in the Junior Village as we have done with the attenuated strains. The naturally occurring virus may be much more *infectious* than the attenuated strains, and therefore may spread more rapidly.

Dr Murray has asked me if the results of the mouse neutralization test can be compared with

those of the tissue culture neutralization test On several occasions in the past we have submitted serum samples to the two tests simultaneously in order to determine the level of antibodies against Type 2 poliovirus Similar results were obtained, and therefore I believe that these tests are comparable

We believe in the use of the most sensitive techniques for the determination of antibodies against poliovirus, provided not too many false positive results would be obtained We consider the immuno-inactivation test of Dr. Gard of particular usefulness in determining levels of antibody Dr Dick does not use this technique and the results he has obtained may be explained by the fact that he employs a less sensitive test, determining, in general, lower levels of antibodies

DR DICK I am not talking about different techniques, I am talking about comparing the first sera with the second sera, tested in one run It is not isolated differences in levels I am talking about, it is a difference in the antibody titer obtained in comparing the level at the beginning of the study with that of the next specimen and the next, all tested at the same time

DR KOPROWSKI I am responsible for the results obtained in our laboratory Dr Dick is responsible for his results, and he can make attempts to explain them

I am not interested in the fact that Dr Dick does not want to draw any conclusions on the duration of immunity after vaccination with live attenuated TN strain of virus I can draw my own conclusion based on scientific facts and not on fanciful hypothesis

The incidence of exposure to three virus types in the course of nine years is probably similar particularly if we consider that this group led a rather sheltered life I have shown data indicating that not more than one third of these subjects developed heterotype antibodies during the nine years of observation Why should we postulate that they have maintained the Type 2 antibody levels because of constant "reinfection" and not because of exposure to the original antigenic stimulus nine years ago It suits Dr. Dick to play with the words "reversion" and "reinfection" in order to make the data on duration of immunity "not acceptable," to him only, I am afraid

If Dr. Dick would check sporadically the antibody levels of his own children whom he fed attenuated virus, he would find, I am sure, that their antibody levels would be maintained for a much longer period of time than those of 197 children who received inactivated virus vaccine

DR. BODIAN: I think the record ought to be clear in relation to Dr Plotkin's comments on Dr. Melnick's discussion

Perhaps Dr Plotkin has misunderstood Dr Melnick's question. I believe that Dr Melnick was referring to the individuals who were contacts of those who were infected with the Cutter material. Such individuals and vaccinated carriers were not infected with attenuated Mahoney They were carrying virulent Mahoney virus

The person in this room to whom I would look for an answer regarding the paralytic capacity of unattenuated Mahoney virus would be Dr Langmuir, whose group made a study of the Cutter vaccine-associated cases. This is pertinent it seems to me, because we have a measure here of the paralytic capacity of a spreading unattenuated strain in an open normal population, as distinct from an institution I would like very much to hear some comment about the statistical aspects of this

DR LANGMUIR: I must admit this is the first time, to my memory, that I have heard the Cutter incident referred to as having been caused by an attenuated strain At the time it occurred, this was certainly not a thinkable concept

The facts are, we had 79 cases among inoculated children, of which 61 were paralytic and 18 non paralytic, we had 105 cases among family contacts of inoculated children, of which 80 were paralytic and 25 were non-paralytic, and among community contacts we had 20 cases, of which 17 were paralytic These 204 cases occurred among somewhere from 300,000 to 400,000 inoculated persons and the family contacts of those inoculated persons, but only two large batches of these vaccines, totalling 124 000 doses, were associated with most of the paralytic cases Some of these doses were retrieved; thus only about 100,000 doses or less were at the "business end" of the Cutter incident

So the rates here are low. Certain figures, I

think, should be placed in the record The attack rate for the most highly infectious batch of Cutter vaccine was 75 per 100,000 doses among inoculated children and the ratio of total associated cases to inoculations given was 155 per 100 000 Thus the hazard to acquiring polio after inoculation with a bad batch, or after family contact with an inoculated child, was less than one in a thousand Thus the question is entirely valid and does focus attention on the problem of measuring the safety of these attenuated strains

I think this is the gravest of all the problems, and so far in my experience, I have seen no test that provides any really satisfactory evidence concerning the relative safety of this vaccine in relation, say, to the safety of the infective Cutter strains

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TOPIC III. PROPERTIES AND BEHAVIOR OF ORALLY ADMINISTERED ATTENUATED STRAINS

1. THE USE OF *IN VITRO* MARKERS AND MONKEY NEUROVIRULENCE TESTS TO FOLLOW GENETIC CHANGES IN ATTENUATED POLIOVIRUS MULTIPLYING IN THE HUMAN ALIMENTARY TRACT *

MATILDA BENYESH-MELNICK AND JOSEPH L. MELNICK

Department of Virology and Epidemiology, Baylor University
College of Medicine, Houston, Texas

DR BENYESH-MELNICK *(presenting the paper)* Perhaps the most important question regarding oral poliovaccine is: How stable genetically are the attenuated strains? The number of tests for monkey neurovirulence carried out with the vaccine viruses after multiplication in the human alimentary tract are few indeed when compared to the number of children who have been fed these viruses

The arbitrary selection of a few specimens for study, out of series of thousands or hundreds of thousands vaccinated, has little significance toward establishing the frequency of genetic change in attenuated polioviruses Under certain circumstances, setting up only a small percentage of carriers of virulent virus might well be dangerous to the vaccinated community or to neighboring communities

Our approach to the problem of genetic stability has been to use *in vitro* tissue culture markers of polioviruses to determine the degree to which these viruses have changed after multiplication in man and to select the altered viruses for the expensive neurovirulence tests Earlier work had demonstrated that certain of the *in vitro* markers of poliovirus have some relationship to neurovirulence

This paper will be concerned chiefly with the *d* (delayed growth in low bicarbonate concentration) [1, 2] and *T* (temperature)[3] markers of Sabin's attenuated Types 1, 2, and 3 polioviruses after multiplication in children vaccinated in 1958 in Mexico City, and the correlation of these markers with monkey neurovirulence

The details of the field study will be reported in a later paper at this Conference It is concerned with 81 families of low socioeconomic level in Mexico City in each of which the youngest child was fed by Dr Ramos Alvarez with approximately 10^7 TCD_{50} of Sabin's polioviruses in sequence, Types 1, 3, and 2, at three week intervals

Rectal swabs were collected before feeding and serially, for 9 weeks after feeding, they were then transported on dry ice to our laboratory at Baylor University College of Medicine in Houston where virus isolations, identifications and characterizations were performed The viruses were all isolated in monkey-kidney cultures maintained in M E medium (0 5% lactalbumin hydrolysate in Earle's salt solution) with 1% monkey serum, 0 15% bovine albumin, and with full 0 22% sodium bicarbonate concentration, the pH of the cultures being 7 4 or above

The first part of this presentation will deal with the results of the *d* character tests obtained with viruses excreted by the vaccinated children and their family contacts Tests for the *d* marker were performed in bottle cultures,[2] using seven

* Aided by a grant from The National Foundation

TABLE 2. *d* CHARACTER TEST PERFORMED ON 7 JAN 1959, WITH STRAINS FROM ORALLY VACCINATED CHILDREN

SPECIMEN NUMBER	LOG₁₀ PFU PER ML		LOG₁₀ EOP*	EOP RATIO TO LSc	CHARACTER
	0 1% BICARB	0 4% BICARB			
635 K_2	<3 0	6 6	>3 6	>1 6	d
1764 K_1	4 2	7 2	3 0	1 4	d
993 K_1	6 3	7 4	1 1	0 5	d±
3046 K_2	6 4	7 2	0 8	0 4	d±
2017 K_1	7 0	7 3	0 3	0 14	d+
3044 K_2	7 4	7 4	0	0	d+
Control LSc	5 2	7 4	2 2	1 0	d
Control Mahoney	8 6	8 6	0	0	d+

* EOP = Efficiency of Plating
d = EOP ratio to LSc > 0 75, d± = EOP ratio to LSc 0.30-0 75.
d+ = EOP ratio to LSc 0-0 29

TABLE 3 RESULTS OF REPEATED *d* CHARACTER TESTS WITH POLIOVIRUSES EXCRETED BY VACCINATED CHILDREN

SPECIMEN NUMBER	DATE OF TEST	LOG₁₀ PFU PER ML AT BICARB CONC		LOG₁₀ EOP	EOP RATIO TO LSc	CHARACTER
		0 1%	0 4%			
1063 K_2	2/ 5/59	<3 0	6 9	>3 9	0 81	d
	2/10/59	<3 0	6 7	>3 7	>0 66	d
	3/ 4/59	3 9	6 7	2 8	1 40	d
2833 K_2	1/20/59	4 4	6 3	1 7	0 80	d
	1/27/59	2 5	6 5	4 0	1 60	d
	5/27/59	5 2	6 5	1 3	0 62	d±
813 K_1	11/22/58	<3 0	7 5	>4 5	0 90	d
	6/ 3/59	5 0	7 0	2 0	0 80	d
635 K_1	1/27/59	<3 0	6 6	>3 6	>1 60	d
	5/27/59	4 2	6 2	2 0	0 95	d
491 K_1	12/ 1/58	<2 0	7 1	>5 1	1 80	d
	6/ 3/59	4 2	6 3	2 1	0 84	d
657 K_1	1/27/59	5 1	7 1	2 0	0 80	d
	5/27/59	4 3	6 4	2 1	1 00	d

TABLE 1. RESULTS OF REPEATED TITRATIONS AT LOW AND HIGH BICARBONATE CONCENTRATIONS WITH *LSc* AND *MAHONEY* STRAINS USED AS CONTROLS IN EACH *d* CHARACTER TEST

DATE OF TEST	LSc			MAHONEY		
	Log_{10} PFU PER ML AT BICARB CONC		Log_{10} EOP	Log_{10} PFU PER ML AT BICARB CONC.		Log_{10} EOP
	0.1%	0.4%		0.1%	0.4%	
1958 8/11	5.5	7.9	2.4	8.6	8.6	0
11/21	<3.0	7.5	>4.5	8.4	8.4	0
12/1	4.6	7.5	2.1	8.6	8.6	0
12/5	5.4	7.9	2.5	8.6	8.7	0.1
12/8	5.2	7.5	2.3	8.5	8.5	0
12/24	3.0	7.2	4.2	8.6	8.5	−0.1
1959 1/7	5.2	7.4	2.2	8.6	8.6	0
1/20	5.6	8.0	2.4	8.4	8.6	0.2
2/5	<3.0	7.8	>4.8	8.5	8.5	0
2/10	<2.0	7.4	>5.4	8.0	8.4	0.4
2/23	5.3	7.3	2.0	8.4	8.3	−0.1
3/4	5.5	7.9	2.4	8.5	8.6	0.1
3/27	5.5	7.7	2.2	8.6	8.7	0.1
4/22	4.5	7.8	3.3	8.4	8.6	0.2
4/27	4.5	7.3	2.8	8.5	8.6	0.1
5/6	5.4	7.4	2.0	8.3	8.3	0
5/12	3.0	7.4	4.4	8.3	8.3	0
5/19	<2.0	7.6	>5.6	7.8	8.0	0.2
5/20	<2.0	7.6	>5.6	8.0	8.1	0.1
6/13	5.2	7.7	2.5	8.1	8.1	0

to nine-day-old monkey kidney monolayers grown and maintained in lactalbumin hydrolysate medium.[4] Parallel virus titrations were performed using 0.1 and 0.4 per cent bicarbonate. The virus inoculum was 0.1 ml. Three bottles were used for each dilution. The virus titers were calculated on the basis of the plaque count on the third day after virus seeding.

LSc and Mahoney strains were used as controls in each *d* character test, and Table 1 represents the results of the repeated titrations of these strains at low (0.1 per cent) and high (0.4 per cent) bicarbonate concentrations. The *d*+ Mahoney virus grew equally well in both bicarbonate concentrations, with negligible variations in the EOP (efficiency of plating) value from test to test. The variations for the *d*− LSc were greater. While its EOP values were usually in the range of 2.0 to 2.5 log_{10} units, in some tests they were much higher (> 5.6 log_{10} units). The latter variation might be due to differences in cell susceptibility of the different lots of monkey kidney cells used, or to other factors beyond our control. Therefore each *d* character test included not only the new strains under study on a particular day, but also the LSc and Mahoney viruses as control *d*− and *d*+ strains.

An example of a *d* character test is illustrated in Table 2. From the titers at the two bicarbonate concentrations, the EOP values were obtained for the test and control viruses (Column 4). Since it was desirable to express our results uniformly throughout the study, several arbitrary values were introduced. The EOP values for the test strains (top 6 lines) and control viruses (bottom 2 lines) were established, and the character of each strain was determined by its EOP ratio to that of LSc, as expressed in the fifth column in this table. The strains tested were separated arbitrarily into three groups

TABLE 5 CORRELATION OF *d* CHARACTER TEST WITH MONKEY NEUROVIRULENCE, TYPE 1 VACCINE AND TYPE 1 STRAINS RECOVERED FROM VACCINATED CHILDREN

Strain	Number Strains Tested	Number of Monkeys with Clinical Poliomyelitis/Number Tested							
		Intraspinal, Log_{10} TCD_{50}						Intracerebral	Rectal Swab IS
		6 3	5 3	4 3	3 3–3 0	2 3–2 0	1 0	7 3–6 2	1 8–1 0
Vaccine fed	1	8/10	4/6	5/6	2/4	0/5		0/12	
Excreted d	4				7/7	0/7		0/3	0/3
Excreted d+	5					10/10	0/8	2/4	2/5
Wild d	1				0/2				0/2

TABLE 6 CORRELATION OF *d* CHARACTER TEST WITH MONKEY NEUROVIRULENCE TYPE 2 VACCINE AND TYPE 2 STRAINS RECOVERED FROM VACCINATED CHILDREN

Strain	Number Strains Tested	Number of Monkeys with Clinical Poliomyelitis/Number Tested							
		Intraspinal, Log_{10} TCD_{50}						Intracerebral	Rectal Swab IS
		6 2	5 2	4 2	3 0	2 0	1 0	7 2–6 2	1 8–1 0
Vaccine fed	1	5/10	4/6	0/6				0/10	
Excreted d	1				0/2			0/2	0/2
Excreted d+	3					6/6	3/5		2/5

$d-$ strains, having an EOP ratio to LSc greater than 0 75, intermediate, or $d\pm$ strains, having an EOP ratio to LSc of 0 30 to 0 75, and $d+$ strains, having an EOP ratio to LSc of 0 to 0 29

The character of each strain was determined on the basis of the results of two and sometimes three tests, the reproducibility of which is illustrated in Tables 2 and 3

Table 3 includes results of repeated tests with several isolates defined as $d-$ strains One can see that although there was variation in the EOP values of some strains, their EOP ratios to those of LSc remained in the same range from test to test, because of simultaneous variations in the EOP values of the LSc strain

In Table 4 are included results of repeated tests with isolates defined as $d+$ strains As with the control Mahoney virus, there was little variation in their EOP values

Representative $d-$ and $d+$ strains were selected for monkey neurovirulence tests, the results of which are summarized in the next four tables

Table 5 represents data on the Type-1 vaccine used and on the Type-1 $d-$ and $d+$ isolates from the vaccinated children. Using the intraspinal route of inoculation, two out of four animals were paralyzed after inoculation with $10^{5\,3}$ TCD_{50} of the vaccine virus, while none developed paralysis when $10^{2\,3}$ TCD_{50} were used By the intracerebral route, the vaccine was free of clinical activity even when $10^{7\,3}$ TCD_{50} were inoculated Four $d-$ isolates were tested, and one can see that their activity in monkeys by both routes of inoculation did not differ significantly from the vaccine strain, which also possessed the $d-$ marker. For the five $d+$ isolates inoculated, a very definite increase in neurovirulence occurred All ten monkeys inoculated intraspinally with 10^2 TCD_{50} developed paralysis, whereas at this concentration of virus the vaccine strain and the other $d-$ strains were completely negative in 12 monkeys Of 4 monkeys inoculated intracerebrally with $10^{6\,2}$ TCD_{50} of the $d+$ strains, two developed paralysis

The increased neurovirulence of these strains

TABLE 4 RESULTS OF REPEATED d CHARACTER TESTS WITH POLIOVIRUSES EXCRETED BY VACCINATED CHILDREN

SPECIMEN NUMBER	DATE OF TEST	LOG_{10} PFU PER ML AT BICARB CONC 0 1%	0 4%	LOG_{10} EOP	EOP RATIO TO LSc	CHARACTER
604 K_1	4/22/59	5 3	7 4	2 1	0 64	d±
	5/12/59	5 0	7 6	1 7	0 39	d±
2251 K_1	8/11/58	7 7	7 3	−0 4	0	d+
	12/ 8/58	7 4	7 5	0 1	0 04	d+
	12/24/58	6 3	7 2	0 9	0 21	d+
2576 K_1	2/23/59	7 4	7 4	0	0	d+
	6/ 3/59	6 7	6 9	0 2	0 08	d+
3461 K_1	3/27/59	6 7	6 9	0 2	0 09	d+
	4/22/59	7 6	7 6	0	0	d+
3485 K_1	3/ 4/59	7 0	7 2	0 2	0 08	d+
	6/ 3/59	6 8	7 3	0 5	0 20	d+
1150 K_1	2/23/59	7 7	7 5	−0 2	0	d+
	6/ 3/59	7 1	7 5	0 4	0 16	d+

TABLE 9 REPRESENTATIVE RESULTS OF *d* CHARACTER TESTS WITH HOMOTYPIC POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

CHILD No	TYPE AND CHARACTER OF VIRUS ISOLATED FROM SPECIMENS									
	PRE-FEEDING	POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
128	0	P1 d	P1 d±	P1 d	0	0	0	P2 d±	P2 d±	—
27	—	P1 d±	P1 d±	0	0	0	0	0	[P2 d]	P2 d±
215	0	P1 d±	P1 d±	0	0	0	0	0	0	[P2 d+]
35	0	[P1 d±]	[P1 d]	0	P3 d+	NP	P3 d+	NP	—	NP
138	0	P1 d	P1 d	P1 d	—	P3 d+	NP	0	0	0
246	0	[P1 d]	P1 d	0	[P3 d]	0	0	—	—	P3 d+
4	0	[P1 d]	P1 d	[P1 d+]	P1 d+	NP	[P3 d+]	P3 d+	—	—
268	0	P1 d	P1 d+	0	0	NP	NP	P2 d	P2 d+	0
107	0	0	0	0	P3 d+	P3 d+	NP	0	P3 d+	0
372	0	P1 d+	[P1 d+]	0	P3 d+	P3 d±	[P3 d+]	0	0	—
376	0	P1 d+	[P1 d+]	0	0	0	NP	—	—	P2 d+
126	0	P1 d+	0	NP	NP	0	NP	[P2 d+]	P2 d+	—
244	0	0	0	0	0	0	0	0	P2 d+	[P2 d+]

P1 = Poliovirus Type 1, P2 = Type 2, P3 = Type 3, NP = Non polio virus, 0 = negative,
— indicates no specimen
d = EOP ratio to LSc > 0.75, d± = EOP ratio to LSc 0.30-0.75, d+ = EOP ratio to LSc 0-0.29
[ ] indicates strain tested in monkeys

TABLE 7. CORRELATION OF *d* CHARACTER TEST WITH MONKEY NEUROVIRULENCE TYPE 3 VACCINE AND TYPE 3 STRAINS RECOVERED FROM VACCINATED CHILDREN

Strain	Number Strains Tested	Number of Monkeys with Clinical Poliomyelitis/Number Tested							
		Intraspinal Log₁₀ TCD₅₀ 6 5	5 5	4 5	3 5–3 0	2 5–2 0	1 0	Intracerebral 7 5–6 2	Rectal Swab IS 1 8–1 0
Vaccine fed	1	9/10	3/6	3/6	0/4	0/4		0/10	
Excreted d	1				2/2	0/2		0/1	0/1
Excreted d+	2					4/4	3/3	2/2	3/3
Wild d+	1					2/2	1/2	2/2	

TABLE 8 CORRELATION OF *d* CHARACTER TESTS WITH MONKEY NEUROVIRULENCE: SUMMARY OF TYPE 1, 2, AND 3 POLIOVACCINES AND POLIO STRAINS RECOVERED FROM VACCINATED CHILDREN

Strain	Number Strains Tested	Number of Monkeys with Clinical Poliomyelitis/Number Tested							
		Intraspinal Log₁₀ TCD₅₀ 6 5–6 2	5 5–5 2	4 5–4 2	3 5–3 0	2 5–2 0	1 0	Intracerebral 7 5–6 2	Rectal Swab IS 1 8–1 0
Vaccines fed	3	22/30	11/18	8/18	2/8	0/9		0/32	
Excreted d	6				9/11	0/9		0/5	0/5
Excreted d+	10					20/20	6/16	4/6	7/13
Wild P1 d	1				0/2				0/2
Wild P3 d+	1					2/2	1/2	2/2	

TABLE 9 REPRESENTATIVE RESULTS OF *d* CHARACTER TESTS WITH HOMOTYPIC POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

CHILD No	PRE-FEEDING	TYPE AND CHARACTER OF VIRUS ISOLATED FROM SPECIMENS: POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
128	0	P1 d	P1 d±	P1 d	0	0	0	P2 d±	P2 d±	—
27	—	P1 d±	P1 d±	0	0	0	0	0	[P2 d]	P2 d±
215	0	P1 d±	P1 d±	0	0	0	0	0	0	[P2 d+]
35	0	[P1 d±]	[P1 d]	0	P3 d+	NP	P3 d+	NP	—	NP
138	0	P1 d	P1 d	P1 d	—	P3 d+	NP	0	0	0
246	0	[P1 d]	P1 d	0	[P3 d]	0	0	—	—	P3 d+
4	0	[P1 d]	P1 d	[P1 d+]	P1 d+	NP	[P3 d+]	P3 d+	—	—
268	0	P1 d	P1 d+	0	0	NP	NP	P2 d	P2 d+	0
107	0	0	0	0	P3 d+	P3 d+	NP	0	P3 d+	0
372	0	P1 d+	[P1 d+]	0	P3 d+	P3 d±	[P3 d+]	0	0	—
376	0	P1 d+	[P1 d+]	0	0	0	NP	—	—	P2 d+
126	0	P1 d+	0	NP	NP	0	NP	[P2 d+]	P2 d+	—
244	0	0	0	0	0	0	0	0	P2 d+	[P2 d+]

P1 = Poliovirus Type 1, P2 = Type 2, P3 = Type 3, NP = Non polio virus, 0 = negative, — indicates no specimen
d = EOP ratio to LSc > 0.75, d± = EOP ratio to LSc 0.30–0.75, d+ = EOP ratio to LSc 0–0.29
[ ] indicates strain tested in monkeys

was even more apparent from the results obtained with virus present in rectal swabs without tissue culture passage. The virus content of the swabs was low, ranging from $10^{1.0}$ to $10^{1.8}$ TCD_{50} per 0.1 ml ; however, this was sufficient to paralyze two of the five monkeys inoculated intraspinally with 0.1 ml of the rectal swab suspensions

In this table we intentionally inserted data on a wild $d-$ strain. This was a wild polio Type-1 virus excreted from a child before he was fed with Type-1 vaccine. It is worth noting that this strain had less intraspinal activity than the vaccine used

Data for Type-2 vaccine and isolates are presented in Table 6

The Type-2 $d+$ isolates from children vaccinated with Type-2 vaccine showed an even greater shift in intraspinal activity than did the Type-1 strains Three out of the five monkeys inoculated with only 10 TCD_{50} of the isolates became paralyzed, while the endpoint of the Type-2 vaccine was reached with $10^{5.2}$ TCD_{50} Thus $10^{5.2}$ TCD_{50} of the vaccine paralyzed four out of six monkeys tested but $10^{4.2}$ TCD_{50} were free of intraspinal activity

Table 7 includes data on Type-3 vaccine and isolates The results are similar to those for Type-2, but even more striking in the reversion to neurovirulence A wild Type-3 $d+$ strain was encountered in a pre-vaccinal specimen and found to have a high degree of monkey neurovirulence

Table 8 summarizes the data of Tables 5 6, and 7, for the three types Although the total number of monkeys used in these tests was relatively small, there is unequivocal evidence of increased intraspinal and intracerebral activity in the $d+$ isolates from vaccinated children in this study.

The cords of all the monkeys inoculated with the rectal swabs were tested for virus in tissue culture They all yielded cytopathogenic virus and in only one cord was a zone phenomenon observed

Representative results of d character tests with homotypic polioviruses excreted by the orally vaccinated children are shown in Table 9 The table includes the code numbers of the children the types of virus isolated in their pre- and post feeding specimens, and the d character of the homotypic polioviruses excreted Homotypic virus indicates poliovirus of the same type as that in the vaccine fed, and excreted after the feeding. The squares around some of the poliovirus isolates indicate those which were tested in monkeys. Further data on the frequency of homotypic virus excretion are given in our subsequent paper at this Conference.

As far as Type-1 isolates are concerned, the first six children represented in Table 9 excreted only $d-$ or $d \pm$ viruses. Children No 4 and No 268, who excreted Type-1 virus for several weeks, revealed a change from $d-$ to $d+$ character in their third and second isolates, respectively On the other hand, children No. 372, No 376, and No 126 excreted $d+$ viruses in the first week after feeding, thus indicating an early alteration of the vaccine virus in the intestinal tract The majority of the Type-3 isolates were of the $d+$ character, while the frequency of change in the Type-2 isolates was similar to that in the Type 1 strains.

Table 10 is presented in order to illustrate the pattern of virus excretion and the character of the heterotypic wild polioviruses excreted by some of the vaccinated children, but unrelated to their ingestion of vaccine virus In the course of the study, 13 heterotypic viruses were isolated from the post-feeding specimens and 2 from the pre-feeding specimens, from a total of five children; all are included in the table We considered as heterotypic the polioviruses isolated from the children before the vaccine virus of the same type had been fed Subsequent isolations of the same type in such a child were also regarded as heterotypic

An example is child No. 65 who failed to exhibit homotypic infection with Types 1 and 3, however he excreted Type-2 poliovirus before feeding with the Type-2 vaccine and continued to excrete it for two more weeks after feeding We considered all these as wild Type-2 viruses, they were all of the $d+$ type

Child No 99 behaved in the same way for Type 3

Child No 102 was negative in his pre-feeding specimen, excreted homotypic Type-1 virus of $d-$ character for two weeks after feeding, and then became infected with wild Type-3 virus before the feeding with the Type 3 vaccine; for two more weeks he continued to excrete what we regarded as a wild Type 3 strain.

Child No 224 excreted Type-3 virus in his pre-

TABLE 10 RESULTS OF *d* CHARACTER TESTS WITH HETEROTYPIC POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

CHILD No.	Type and Character of Virus Isolated from Specimens									
	PRE-FEEDING	POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
65	0	0	0	—	0	—	P2 d+	P2 d+	P2 d+	NP
99	NP	NP	NP	P3 d+	P3 d+	P3 d+	0	NP	NP	—
102	0	[P1 d]	[P1 d]	[P3 d+]	P3 d+	P3 d+	0	0	NP	—
224	P3 d+	P3 d+	NP	NP	NP	P3 d+	0	0	—	0
380	[P1 d]	0	0	NP	NP	0	0	P1 d	P1 d	—

P1 = Poliovirus Type 1; P2 = Type 2, P3 = Type 3, NP = Non polio virus, 0 = negative,
— indicates no specimen
d = EOP ratio to LSc > 0 75, d± = EOP ratio to LSc 0 30-0 75, d+ = EOP ratio to LSc 0 0.29
[ ] indicates strain tested in monkeys

TABLE 11 CHARACTER OF POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

TIME AFTER FEEDING (DAYS)	TYPE 1				TYPE 2				TYPE 3			
	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+
7	20	11	5	4	13	2	3	8	5	1	0	4
14	16	6	5	5	7	1	0	6	5	0	1	4
21	3	2	0	1	5	0	1	4	5	0	0	5
28	No specimens				No specimens				4	0	0	4
35	No specimens				No specimens				1	0	0	1
42	No specimens				No specimens				3	0	0	3
56	No specimens				No specimens				No specimens			
Total	39	19	10	10	25	3	4	18	23	1	1	21

d indicates EOP (Efficiency of Plating) ratio to LSc > 0 75
d± indicates EOP ratio to LSc 0.30-0 75
d+ indicates EOP ratio to LSc 0-0.29

was even more apparent from the results obtained with virus present in rectal swabs without tissue culture passage The virus content of the swabs was low, ranging from $10^{1.0}$ to $10^{1.8}$ TCD_{50} per 0 1 ml, however, this was sufficient to paralyze two of the five monkeys inoculated intraspinally with 0 1 ml. of the rectal swab suspensions

In this table we intentionally inserted data on a wild $d-$ strain This was a wild polio Type 1 virus excreted from a child before he was *fed with Type-1 vaccine It is worth noting that* this strain had less intraspinal activity than the vaccine used

Data for Type-2 vaccine and isolates are presented in Table 6

The Type-2 $d+$ isolates from children vaccinated with Type-2 vaccine showed an even greater shift in intraspinal activity than did the Type-1 strains Three out of the five monkeys inoculated with only 10 TCD_{50} of the isolates became paralyzed while the endpoint of the Type-2 vaccine was reached with $10^{5.2}$ TCD_{50} Thus $10^{5.2}$ TCD_{50} of the vaccine paralyzed four out of six monkeys tested but $10^{4.2}$ TCD_{50} were free of intraspinal activity

Table 7 includes data on Type-3 vaccine and isolates The results are similar to those for Type-2, but even more striking in the reversion to neurovirulence A wild Type-3 $d+$ strain was encountered in a pre vaccinal specimen and found to have a high degree of monkey neurovirulence

Table 8 summarizes the data of Tables 5 6 and 7, for the three types Although the total number of monkeys used in these tests was relatively small, there is unequivocal evidence of increased intraspinal and intracerebral activity in the $d+$ isolates from vaccinated children in this study.

The cords of all the monkeys inoculated with the rectal swabs were tested for virus in tissue culture. They all yielded cytopathogenic virus and in only one cord was a zone phenomenon observed.

Representative results of d character tests with homotypic polioviruses excreted by the orally vaccinated children are shown in Table 9 The table includes the code numbers of the children, the types of virus *isolated in their pre-* and post feeding specimens, and the d character of the homotypic polioviruses excreted Homotypic virus indicates poliovirus of the same type as that in the vaccine fed, and excreted after the feeding. The squares around some of the polio-virus isolates indicate those which were tested in monkeys. Further data on the frequency of homotypic virus excretion are given in our subsequent paper at this Conference.

As far as Type-1 isolates are concerned, the first six children represented in Table 9 excreted only $d-$ or $d \pm$ viruses. Children No 4 and No 268, who excreted Type-1 virus for several weeks, revealed a change *from $d-$ to $d+$ charac*ter in their third and second isolates, respectively On the other hand, children No 372, No 376 and No. 126 excreted $d+$ viruses in the first week after feeding, thus indicating an early alteration of the vaccine virus in the intestinal tract The majority of the Type-3 isolates were of the $d+$ character, while the frequency of change in the Type-2 isolates was similar to that in the Type-1 strains

Table 10 is presented in order to illustrate the pattern of virus excretion and the character of the heterotypic wild polioviruses excreted by some of the vaccinated children, but unrelated to their ingestion of vaccine virus In the course of the study, 13 heterotypic viruses were isolated from the post-feeding specimens and 2 from the pre-feeding specimens, from a total of five children; all are included in the table. We considered as heterotypic the polioviruses isolated from the children before the vaccine virus of the same type had been fed Subsequent isolations of the same type in such a child were also regarded as heterotypic

An example is child No 65, who failed to exhibit homotypic infection with Types 1 and 3, however, he excreted Type-2 poliovirus before feeding with the Type-2 vaccine and continued to excrete it for two more weeks after feeding We considered all these as wild Type-2 viruses, they were all of the $d+$ type

Child No 99 behaved in the same way for Type 3

Child No 102 was negative in his pre-feeding specimen, excreted homotypic Type-1 virus of $d-$ character for two weeks after feeding, and then became infected with wild Type-3 virus before the feeding with the Type 3 vaccine; for two more weeks he continued to excrete what we regarded as a wild Type-3 strain

Child No 224 excreted Type-3 virus in his pre-

TABLE 10. RESULTS OF *d* CHARACTER TESTS WITH HETEROTYPIC POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

CHILD No	TYPE AND CHARACTER OF VIRUS ISOLATED FROM SPECIMENS									
	PRE-FEEDING	POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
65	0	0	0	—	0	—	P2 d+	P2 d+	P2 d+	NP
99	NP	NP	NP	P3 d+	P3 d+	P3 d+	0	NP	NP	—
102	0	[P1 d]	[P1 d]	[P3 d+]	P3 d+	P3 d+	0	0	NP	—
224	P3 d+	P3 d+	NP	NP	NP	P3 d+	0	0	—	0
380	[P1 d]	0	0	NP	NP	0	0	P1 d	P1 d	—

P1 = Poliovirus Type 1, P2 = Type 2, P3 = Type 3, NP = Non polio virus, 0 = negative,
— indicates no specimen
d = EOP ratio to LSc > 0 75, d± = EOP ratio to LSc 0 30 0 75, d+ = EOP ratio to LSc 0 0 29
[ ] indicates strain tested in monkeys

TABLE 11 CHARACTER OF POLIOVIRUSES EXCRETED BY ORALLY VACCINATED CHILDREN

TIME AFTER FEEDING (DAYS)	TYPE 1				TYPE 2				TYPE 3			
	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+
7	20	11	5	4	13	2	3	8	5	1	0	4
14	16	6	5	5	7	1	0	6	5	0	1	4
21	3	2	0	1	5	0	1	4	5	0	0	5
28	No specimens				No specimens				4	0	0	4
35	No specimens				No specimens				1	0	0	1
42	No specimens				No specimens				3	0	0	3
56	No specimens				No specimens				No specimens			
Total	39	19	10	10	25	3	4	18	23	1	1	21

d indicates EOP (Efficiency of Plating) ratio to LSc > 0 75
d± indicates EOP ratio to LSc 0 30 0 75
d+ indicates EOP ratio to LSc 0 0 29

feeding specimen and thereafter for several weeks.

Child No 380 excreted Type-1 virus of *d*— character in his pre-feeding specimen. Seven weeks later the child excreted for two more weeks a virus of the same type and character. With this exception, all of the wild heterotypic viruses were of the *d*+ character

The *d* character of all the homotypic polioviruses tested in this study is shown in Table 11 Of 39 Type-1 viruses tested, 19 remained unchanged, that is, *d*— in character. Ten changed to *d*±: and 10 changed to *d*+ Of 25 Type-2 viruses tested, only 3 were *d*—; 4 were *d*±, and 18 were *d*+ The change was even greater for the Type 3 viruses—only one of the 23 strains tested remained *d*—, one changed to *d*±, and 21 changed to *d*+

From the foregoing, it is apparent that the interpretation of data obtained with isolates of children vaccinated in a naturally polio-infected population, such as the one studied, is difficult indeed It becomes even more difficult to try to interpret the results with the strains isolated from family contacts of the children fed For example, in Table 12, let us consider Family A, in which child No 4 (the underlining indicates the "index child"), a six-month-old baby, was fed the three vaccine viruses and excreted homotypic Type-1 and Type-3 viruses One of the contacts excreted Type-3 virus It seems to us that this virus was excreted at such a time as to be appropriately considered the result of contact infection with the progeny of the vaccine virus from the index child The virus excreted by the vaccinated baby was *d*+, the virus excreted by the contact was *d*+ also Similar findings were obtained from a study of Family B

In Family C, child No 41 was fed, and homotypic Type-3 poliovirus was detected only in the specimen obtained 3 weeks after feeding One of the siblings, a four-year-old child, excreted Type-3 virus two weeks before we detected it in the vaccinated child It might very well have been that we failed to detect virus in the first specimen from the child fed, but that he had been infected with the vaccine virus, and that he transmitted it to his sibling during the seven-day post-feeding period On the other hand, there is a possibility that the index child became infected with a wild Type-3 poliovirus being excreted by his sibling in the course of a natural infection. The same speculation could be elaborated for the Type-1 poliovirus excreted by the mother of the same child In some of the families (E, F, and G), poliovirus was isolated from family contacts but not from the index children themselves Almost all of the viruses isolated from the family contacts were *d*+ strains, as is shown in Table 13 Fourteen Type-1 strains were tested One was of the intermediate *d*± character, and 13 were *d*+. Only one Type 2 strain was isolated from the contacts, and it was a *d*+ virus There were eight Type-3 strains tested, seven were *d*+, and one was *d*±

Results of the T character tests

The following tables deal with the results obtained with isolates from vaccinated children when tested for the newly introduced *T* marker Parallel virus titrations were performed under two conditions: using 37°C, and 40°C, for the incubation of the inoculated cultures. The temperature variation of the 40°C. incubation was ± 0 5°C.

Table 14 lists results obtained for Mahoney virus in repeat tests CPE titers were estimated on days 3, 5, 7, 9, and 11 of the test The differences in titers obtained at the two temperatures were expressed as $\log_{10}$ EOG (efficiency of growth) values ($\log_{10}$ EOG = log TCD_{50} per ml at 37°C. minus log TCD_{50} per ml at 40°C.) For Mahoney, the 3-day EOG values ranged between 0.7 and 3 0 $\log_{10}$ units They decreased with time and by the seventh day were as low as 0 3 to 1 0 $\log_{10}$ units No additional changes occurred after the seventh day, therefore all our calculations with test viruses were based on the seventh day CPE reading values.

Table 15 includes the value obtained for parallel tests with LSc virus. A striking difference was noted between Mahoney and LSc strains, for with the latter the efficiency of growth at the seventh day was always more than 6 $\log_{10}$ units, with negligible variation from test to test

We calculated our *T* character data with the test viruses similarly to the calculations for the *d* character tests Table 16 illustrates a *T* character test performed on 3 June 1959, with isolates from vaccinated children. The virus titers and EOG values were determined on the seventh day of the experiment. Since Mahoney usually gave an EOG value of up to one $\log_{10}$ unit, we sub

TABLE 12 RESULTS OF *d* CHARACTER TESTS WITH POLIOVIRUSES EXCRETED BY VACCINATED CHILDREN AND SOME OF THEIR FAMILY CONTACTS

FAMILY	INDIV No	AGE	TYPE AND CHARACTER OF VIRUS ISOLATED FROM SPECIMENS								
			POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
			7 da	14 da	21 da	7 da	14 da	21 da	7 da	14 da	21 da
A	*4**	6 mos	P1 d	P1 d	P1 d+	P1 d+	NP	P3 d+	P3 d+	—	—
	9	8 yrs	0	0	0	0	NP	0	P3 d+	—	—
B	*219*	2 yrs	0	0	0	0	0	P3 d+	P3 d+	0	P3 d+
	221	5 yrs	0	0	0	0	0	0	P3 d+	0	0
C	*41*	14 mos	0	NP	—	0	NP	P3 d+	0	0	NP
	40	24 yrs	0	0	0	0	0	0	0	0	P1 d+
	42	4 yrs	0	0	0	P3 d+	NP	NP	NP	0	NP
D	*17*	4 mos	NP	NP	NP	NP	0	0	P2 d+	P2 d+	NP
	18	2 yrs	0	0	0	0	0	0	0	P1 d+	0
	19	5 yrs	0	0	P1 d+	0	0	0	0	0	0
E	*44*	1 mo	0	0	—	0	0	—	0	0	0
	43	34 yrs	0	0	P1 d+	—	P1 d+	0	NP	0	0
	45	12 mos	0	0	0	0	0	—	P3 d+	P3 d+	P1 d+
F	*75*	2 yrs	0	0	—	NP	NP	NP	—	—	—
	76	5 yrs	0	P1 d+	P1 d—	P1 d+	P1 d+	P1 d+	—	—	—
G	*116*	4 mos	0	0	0	0	0	0	NP	0	0
	117	3 yrs	0	0	P1 d+	0	0	NP	0	NP	0
	118	5 yrs	0	0	0	P1 d+	0	NP	NP	0	0

* Italics indicates child vaccinated

TABLE 13 CHARACTER OF POLIOVIRUSES EXCRETED BY FAMILY CONTACTS OF ORALLY VACCINATED CHILDREN

TIME AFTER FEEDING* (DAYS)	TYPE 1				TYPE 2				TYPE 3			
	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+	NUMBER TESTED	d	d±	d+
7	No specimens				No specimens				2	0	0	2
14	1	0	0	1	No specimens				1	0	0	1
21	4	0	0	4	1	0	0	1	1	0	0	1
28	2	0	0	2	No specimens				3	0	1	2
35	2	0	0	2	No specimens				1	0	0	1
42	2	0	1	1	No specimens				No specimens			
56	3	0	0	3	No specimens				No specimens			
Total	14	0	1	13	1	0	0	1	8	0	1	7

* [illegible]

** ratio to LSc > 0.75

TABLE 14 RESULTS OF REPEATED TUBE TITRATIONS AT 37°C AND 40°C WITH MAHONEY STRAIN USED AS CONTROL IN EACH t CHARACTER TEST

DATE OF TEST	Log_{10} TCD$_{50}$ PER ML ON DAYS OF READING INDICATED								Log_{10} EOG* ON DAYS OF READING INDICATED			
	At 37°C				At 40°C							
	3	5	7	9–11	3	5	7	9–11	3	5	7	9–11
1959:												
5/13	7.5	8.2	8.2	8.2	<5.0	8.2	8.5	8.5	>2.5	0	−0.3	−0.3
5/19	8.2	8.5	8.8	8.8	7.5	7.8	8.5	8.5	0.7	0.7	0.3	0.3
5/20	7.2	8.8	8.8	8.8	5.5	8.2	8.5	8.5	1.7	0.6	0.3	0.3
5/26	7.5	8.2	8.5	8.5	5.5	5.5	7.5	7.8	2.0	2.7	1.0	0.7
5/27	6.8	7.8	8.2	8.2	<4.0	<4.0	7.2	7.2	>2.8	>3.8	1.0	1.0
5/28	6.5	8.2	8.2	8.2	5.5	7.2	7.5	8.2	1.0	1.0	0.7	0.7
6/2	7.2	7.5	7.8	7.8	4.8	7.5	7.8	7.8	2.6	0	0	0
6/3	6.5	7.5	8.2	8.2	<4.0	7.2	7.2	7.5	>2.5	0.3	1.0	0.7
6/5	8.2	8.5	8.5	8.5	5.2	7.2	7.5	7.5	3.0	1.3	1.0	1.0

* Log_{10} FOG = Log_{10} Efficiency of Growth
= Log TCD$_{50}$ per ml. at 37°C.—Log TCD$_{50}$ per ml. at 40°C.

TABLE 15 RESULTS OF REPEATED TUBE TITRATIONS AT 37°C. AND 40°C. WITH LSc STRAINS USED AS CONTROL IN EACH *t* CHARACTER TEST

DATE OF TEST	Log_{10} TCD$_{50}$ PER ML ON DAYS OF READING INDICATED						Log_{10} EOG* ON DAYS OF READING INDICATED			
	AT 37°C				AT 40°C					
	3	5	7	9-11	3-5	7-11	3	5	7	9-11
1959										
5/13	4 7	7 2	7 8	7 8	<1	<1	>3 7	>6 2	>6 8	>6 8
5/19	6 2	6 8	7 8	7 8	<1	<1	>5 2	>5 8	>6 8	>6 8
5/20	5 5	7 5	7 5	7 5	<1	<1	>4 5	>6 5	>6 5	>6 5
5/26	5 5	7 8	8 5	8 5	<1	<1	>4 5	>6 8	>7 5	>7 5
5/27	4 5	7 5	7 5	7 5	<1	<1	>3 5	>6 5	>6 5	>6 5
5/28	6 0	7 2	7 8	7 8	<1	<1	>5 0	>6 2	>6 8	>6 8
6/2	5 5	7 5	7 8	7 8	<1	<1	>4 5	>6 5	>6 8	>6 8
6/3	4 5	6 8	7 5	8 2	<1	<1	>3 5	>5 8	>6 5	>7 2
6/5	6 8	8 2	8 2	8 2	<1	<1	>5 8	>7 2	>7 2	>7 2

* Log_{10} EOG = Log_{10} Efficiency of Growth
= Log TCD$_{50}$ per ml at 37°C − Log TCD$_{50}$ per ml at 40°C

TABLE 16 *t* CHARACTER TEST PERFORMED ON 3 JUNE, 1959, WITH STRAINS FROM ORALLY VACCINATED CHILDREN

SPECIMEN NUMBER	Log_{10} TCD$_{50}$ PER ML IN DAY 7 READING AT		Log_{10} EOG*	Log_{10} EOG CORRECTED FOR MAHONEY	EOG RATIO TO LSc	CHARACTER
	37°C	40°C				
2453 K_1	6 8	>5 5	<1 3	<0 3	<0 05	t+
2799 K_1	7 5	>5 5	<2 0	<1 0	<0 15	t+
2801 K_1	7 5	>6 5	<1 0	0	0	t+
2597 K_1	6 2	2 5	3 7	2 7	<0 42	t±
2638 K_1	8 3	4 7	3 6	2 6	<0 40	t±
657 K_1	6 8	<1 0	>5 8	>4 8	1 00	t
2945 K_1	7 2	<1 0	>6 2	>5 2	1 00	t
1063 K_1	6 8	<1 0	>5 8	>4 8	1 00	t
Control LSc	7 5	<1 0	>6 5	>5 5	1 00	t
Control Mah	8 5	7 5	1 0	0	0	t+

* EOG = Efficiency of Growth
t = EOG ratio to LSc > 0 75, t± = EOG ratio to LSc 0 30 0 75, t+ = EOG ratio to LSc 0-0 29

tracted its EOG value from the EOG values of all the viruses included in the test We arbitrarily determined the T value of a strain by its EOG ratio to that of LSc Here again the strains tested were separated arbitrarily into three groups T-strains—strains which had an EOG ratio to LSc greater than 0 75, intermediate or $T\pm$ strains—those having an EOG ratio to LSc of 0 30 to 0 75, and $T+$ strains—with an EOG ratio to LSc of 0 to 0 29

The T character of each strain was usually determined on the basis of the results of two consecutive tests The reproducibility of the test is illustrated in Tables 17 and 18. The tables include representative results of repeated T character tests with the original Type 1, 2, and 3 vaccine viruses, and with some $d-$ and $d+$ isolates from vaccinated children While all the $d-$ strains were $T-$ also, not all the $d+$ altered strains exhibited changes in the same direction for the T marker As seen in Table 18, some $d+$ isolates (3167, 2710, and 2251) remained unchanged for their T marker.

Results of the same nature are illustrated in Table 19. This table represents parallel d and T character tests run on the same day, using the same virus dilutions and cell stock. While in isolates 2576 and 1607 the reversion from $d-$ to $d+$ was associated with a reversion from $T-$ to $T+$ also, this was not so for the following two isolates The were of the $d+T-$ character The last two isolates in this table retained the unchanged $d-T-$ markers of the vaccine administered

In Table 20 are shown the results of d and T character tests with 36 homotypic polio strains recovered from vaccinated children. In 11 isolates there was no detectable alteration in the virus, in 19 others, the recovered virus changed in the d but not in the T marker; but in 6 changes had occurred in both markers Thus in 17% of the children infected with $d-T-$

TABLE 17 REPEATED t CHARACTER TESTS WITH THE ORIGINAL VACCINE VIRUS AND WITH A d AND A $d+$ VIRUS ISOLATED FROM VACCINATED CHILDREN

Specimen Number	Date of Test (1959)	Log_{10} TCD_{50} per ml in Day 7 Reading at 37°C	40°C	Log_{10} EOG Corrected for Mahoney*	EOG Ratio to LSc	t Character	d Character Determined in Previous Tests
Vaccine Polio-1	5/20	7 5	<1 0	>6 2	1	t	
	5/28	7 8	<1 0	>6 1	1	t	d
	6/3	7 2	<1 0	>6 2	1	t	
Vaccine Polio-2	5/20	6 8	<1 0	>5 2	1	t	
	5/28	7 2	<1 0	>5 5	1	t	d
	6/3	7 2	<1 0	>5 2	1	t	
Vaccine Polio-3	5/20	6 7	<1 0	>5 4	1	t	
	5/28	7 2	<1 0	>5 5	1	t	d
	6/3	7 2	<1 0	>5 2	1	t	
657 K_1	5/27	6 8	<1 0	>4 8	1	t	d
	6/3	6 8	<1 0	>4 8	1	t	
2453 K_1	5/27	6 5	5 8	−0 3	0	t+	d+
	6/3	6 8	>5 5	<0 3	<0 05	t+	

* Log_{10} EOG for Mahoney on 20 May, was 0.3, on 26 May, 1 0, on 27 May, 1.0, on 28 May, 0 7, on 3 June, 1 0

TABLE 18. REPEATED *t* CHARACTER TESTS WITH *d*+ AND *d* VIRUSES ISOLATED FROM VACCINATED CHILDREN

SPECIMEN NUMBER	DATE OF TEST (1959)	Log_{10} TCD_{50} PER ML IN DAY 7 READING AT 37°C	40°C	Log_{10} EOG CORRECTED FOR MAHONEY	EOG RATIO TO *LSc*	*t* CHARACTER	*d* CHARACTER DETERMINED IN PREVIOUS TESTS
2799 K_1	5/27	6 5	5 8	−0 3	0	t+	
	6/3	6 8	>5 5	<1 0	<0 15	t+	d+
2801 K_1	5/27	7 5	6 5	0	0	t+	
	6/3	7 5	>6 5	0	0	t+	d+
2576 K_1	5/26	7 4	6 9	−0 5	0	t+	
	6/3	7 9	6 9	0	0	t+	d+
3167 K_2	5/27	6 8	<1 0	>4 8	1	t	
	6/3	6 8	<1 0	>4 8	1	t	d+
2710 K_2	5/26	7 0	<1 0	>5 0	1	t	d+
	6/3	7 7	<1 0	>5 7	1	t	
2251 K_1	5/26	7 2	<1 0	>5 2	1	t	
	6/3	7 5	<1 0	>5 5	1	t	d+

strains, the excreted virus had changed in at least two properties The *d*− to *d*+ reversion was not always associated with a change from *T*− to *T*+ However, every instance of *T*− to *T*+ reversion was linked with a *d*− to *d*+ reversion. *Of* the 17 strains recovered from family contacts, 14 were *d*+*T*+, one was *d*+*T*±, and one *d*+*T*−

Data on the correlation of the *d* and *T* characters and monkey neurovirulence of the three poliovirus types isolated from the vaccinees are shown in Table 21 It is apparent that excreted strains which retain the *d*−*T*− character of the vaccine virus were not significantly more virulent for monkeys than the vaccines themselves—about 10^4 TCD_{50} being required to paralyze 50% of the monkeys inoculated intraspinally, and no illness being produced either by undiluted tissue culture fluid inoculated intracerebrally, or by rectal swabs inoculated intraspinally The *d*+*T*− showed increased activity, both intraspinally and intracerebrally, when compared to the *d*−*T*− vaccine virus and the *d*−*T*− isolates Thus, only 10 TCD_{50} of the cultured virus were found to contain one monkey paralyzing $dose_{50}$ by the intraspinal route, and some of the strains were also active after intracerebral inoculation Furthermore, only 10^{1} to $10^{1.8}$ TCD_{50} of virus in the rectal swabs themselves produced paralysis in 3 of 9 test monkeys. The strains which changed in both markers, to *d*+*T*+, showed even greater neurovirulence Cytopathogenic virus was recovered from spinal cords of almost all monkeys sacrificed soon after the onset of paralysis, indicating that no selection of strictly neurotropic virus had taken place in the CNS of the monkeys

A Type-3 strain, recovered from a two month-old vaccinated child, had reverted to the *d*+*T*+ state (see history of strain in Table 22) This strain was selected for inoculation into a chimpanzee, as Sabin has shown that the members of this species are much less sensitive to attenuated poliovirus than monkeys.

As seen in Table 23, when undiluted tissue culture fluid was inoculated intraspinally in chimpanzee Delta, it produced complete paralysis of both legs and right arm, with severe

TABLE 19 RESULTS OF PARALLEL *d* AND *t* CHARACTER TESTS PERFORMED ON 3 JUNE 1959 WITH 8 POLIO STRAINS EXCRETED BY VACCINATED CHILDREN

Specimen Number	Log PFU/ml at Bicarb Conc		Log EOP	Log EOP Ratio to LSc	Character	Log TCD_{50}/ml at		Log EOG Corrected for Mah	Log EOG Ratio to LSc	Character
	0 1%	0 4%				37°C	40°C			
2576 K_1	6 7	6 9	0 2	0 08	d+	7 9	6 9	0	0	t+
1697 K_1	7 9	7 8	−0 1	0	d+	7 7	7 7	−1 0	0	t+
1150 K_1	7 1	7 5	0 4	0 16	d+	7 0	<1 0	>5 0	1	t
1154 K_1	7 5	7 8	0 3	0 12	d+	7 0	<1 0	>5 0	1	t
124 K_1	4 6	7 1	2 5	1 0	d	6 8	<1 0	>4 8	1	t
491 K_1	4 2	6 3	2 1	0 84	d	6 2	<1 0	>4 2	1	t

Table 20 Character of Polioviruses Excreted by Vaccinated Children and Family Contacts as Determined by the *d* and *t* Character Tests

Viruses Excreted	Vaccinated Children						Family Contacts			
	Total Tested	d t	d± t	d+ t	d+ t±	d+ t +	Total Tested	d+ t	d+ t±	d+ t+
Homotypic Polio–1	16	8	4	4	0	0	11	0	0	11
Polio–1	8	2	0	6	0	0	1	1	0	0
Polio–3	12	1	0	5	1	5	5	0	2	3
Total	36	11	4	15	1	5	17	1	2	14

Table 21 Correlation of *d* and *t* Characters With Monkey Neurovirulence

Strains	Number Strains Tested	Characters*		Number of Monkeys With Clinical Poliomyelitis/Number Tested					
		d	t	Log_{10} TCD_{50} Intraspinal				Intra-Cerebral	Rectal Swab IS
				4 5–4 2	3 5–3 0	2 5–2 0	1 0	7 5–6 2	1 8–1 0
Vaccines fed	3	−	−	8/18	2/8	0/9		0/32	
Viruses excreted	6	−	−		9/11	0/9		0/2	0/6
	1	±	−			2/2			2/2
	8	+	−		2/2	16/16	4/13	2/6	3/9
	1	+	+			2/2	2/2	2/2	2/2†

* The d and t characters were each established by repeat tests

† Rectal swab inoculated into these animals had t+ character, all other rectal swabs were t No d character tests were performed with racial swab.

histological lesions in the lumbar region and substantial lesions in the thoracic and cervical regions of the spinal cord. However, chimpanzee Gamma, treated in the same manner with the Type-3 vaccine virus, was free of clinical illness and histological changes in the spinal cord. Following are some photomicrographs of the spinal cord of the paralyzed chimpanzee, as compared with the one inoculated with the Type 3 vaccine virus. Chimpanzee Gamma, which was inoculated with Type 3 vaccine, exhibited needle tract damage in the lumbar cord, but no other significant lesions, even at the level of the site of injection (Figure 1). Chimpanzee Delta was inoculated with the reverted *d+T+* Type-3 isolate. Needle tract lesions were also present in the lumbar cord of this chimpanzee, but surrounding the needle tract and spreading to the opposite side of the cord were extensive evidences of poliovirus multiplication (Figure 2). Spread occurred to other levels also, as shown by the extensive polio lesions present in the other levels of the lumbar area (Figure 3) and even in the cervical area (Figure 4).

TABLE 22 HISTORY OF MEXICO SPECIMEN NUMBER 2576 K_1, EXCRETED BY A TWO-MONTH-OLD CHILD, AND SELECTED FOR CHIMPANZEE NEUROVIRULENCE TEST

Type and Character of Vaccine Virus Fed	Type and Character of MK_1 Passage of Virus Excreted On Post-Feeding Day		
	7	14	21
Polio 1 d t	Polio 1 d+	Polio 1 d+ t	0
Polio 3 d t	Polio 3 d+	Polio 3 d±	Polio 3 d+ t+ (Spec. No. 2576 K_1)
Polio 2 d t	0	0	No specimen

TABLE 23. CHIMPANZEE INTRASPINAL NEUROVIRULENCE OF SABIN'S TYPE 3 STRAIN BEFORE FEEDING AND AFTER MULTIPLICATION IN A TWO-MONTH-OLD CHILD

Chimpanzee	Inoculum	Disease	Polio Lesions			Needle Tract Lesions in Lumbar Cord
			Cervical	Thoracic	Lumbar	
Gamma	Sabin's Type 3 Strain	Neg	0	0	5 (0/++)	1, 8
Delta	Mexico Specimen No. 2576 K_1	Complete paralysis of both legs, and right arm	40 (0/+++)	26 (0/+++)	68 (++/++++)	2, 10

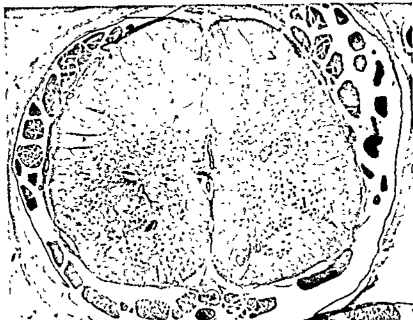


FIG 1 Chimpanzee Gamma, inoculated with Sabin's Type 3 vaccine strain Needle tract lesion in lumbar area of spinal cord The lesion is confined to the left side, the site of the injection, with no evidence of spread to the right hemisection

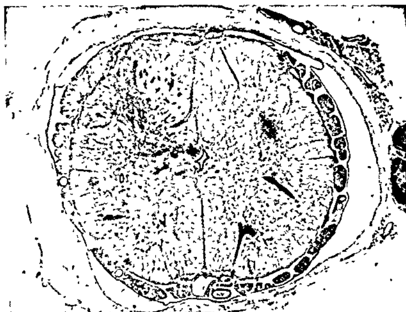


FIG 2 Chimpanzee Delta, inoculated with virus recovered from an infant 21 days after feeding Sabin's Type 3 vaccine The site of the needle damage indicates that the virus was deposited in the left posterior horn The distribution of polio myelitis lesions indicates the spread of virus to the opposite side of the cord

histological lesions in the lumbar region and substantial lesions in the thoracic and cervical regions of the spinal cord However, chimpanzee Gamma, treated in the same manner with the Type-3 vaccine virus, was free of clinical illness and histological changes in the spinal cord. Following are some photomicrographs of the spinal cord of the paralyzed chimpanzee, as compared with the one inoculated with the Type 3 vaccine virus Chimpanzee Gamma, which was inoculated with Type 3 vaccine, exhibited needle tract damage in the lumbar cord, but no other significant lesions, even at the level of the site of injection (Figure 1). Chimpanzee Delta was inoculated with the reverted *d*+*T*+ Type-3 isolate Needle tract lesions were also present in the lumbar cord of this chimpanzee, but surrounding the needle tract and spreading to the opposite side of the cord were extensive evidences of poliovirus multiplication (Figure 2). Spread occurred to other levels also, as shown by the extensive polio lesions present in the other levels of the lumbar area (Figure 3) and even in the cervical area (Figure 4).

TABLE 22. HISTORY OF MEXICO SPECIMEN NUMBER 2576 K_1, EXCRETED BY A TWO-MONTH OLD CHILD, AND SELECTED FOR CHIMPANZEE NEUROVIRULENCE TEST

Type and Character of Vaccine Virus Fed	Type and Character of MK_1 Passage of Virus Excreted On Post-Feeding Day		
	7	14	21
Polio 1 d t	Polio 1 d+	Polio 1 d+ t	0
Polio 3 d t	Polio 3 d+	Polio 3 d±	Polio 3 d+ t+ (Spec No 2576 K_1)
Polio 2 d t	0	0	No specimen

TABLE 23 CHIMPANZEE INTRASPINAL NEUROVIRULENCE OF SABIN'S TYPE 3 STRAIN BEFORE FEEDING AND AFTER MULTIPLICATION IN A TWO-MONTH-OLD CHILD

Chimpanzee	Inoculum	Disease	Polio Lesions			Needle Tract Lesions in Lumbar Cord
			Cervical	Thoracic	Lumbar	
Gamma	Sabin's Type 3 Strain	Neg	0	0	5 (0/++)	1, 8
Delta	Mexico Specimen No 2576 K_1	Complete paralysis of both legs, and right arm	40 (0/+++)	26 (0/+++)	68 (++/++++)	2, 10

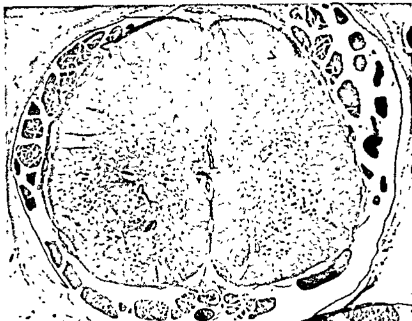


Fig 1 Chimpanzee Gamma, inoculated with Sabin's Type 3 vaccine strain Needle tract lesion in lumbar area of spinal cord The lesion is confined to the left side, the site of the injection, with no evidence of spread to the right hemisection

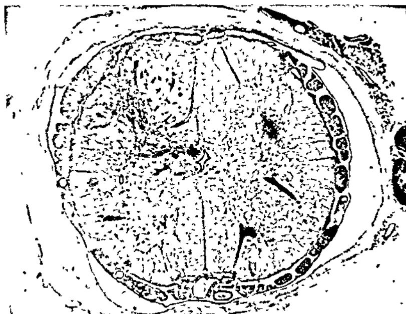


Fig 2 Chimpanzee Delta, inoculated with virus recovered from an infant 21 days after feeding Sabin's Type 3 vaccine The site of the needle damage indicates that the virus was deposited in the left posterior horn The distribution of poliomyelitis lesions indicates the spread of virus to the opposite side of the cord



FIG 3 Chimpanzee Delta Another level of the lumbar cord away from the site of the injection No evidence of the needle tract can be seen at this level, which shows severe lesions produced by poliovirus multiplication



FIG 4 Chimpanzee Delta. Poliomyelitis lesions in the cervical cord, far removed from the inoculation site

SUMMARY AND CONCLUSIONS

The degree of genetic stability of the attenuated virus as it undergoes multiplication cycles in the intestinal tract of vaccinated children and their contacts is of the utmost importance The *use of tissue culture markers for detecting* genetic alterations in attenuated strains used in field studies has been illustrated in our study of Sabin's strains fed to children in Mexico City The excreted viruses which showed the greatest alteration in the *in vitro d* and *T* tissue culture markers also showed the greatest increase in monkey neurovirulence, exhibiting intracerebral activity and also marked intraspinal activity with only a few viral particles One altered strain was also found to be paralytogenic for a chimpanzee

That the increased monkey neurovirulence was not a laboratory artifact, brought on by cultivation of the virus under conditions favoring the growth of virulent particles, is shown by the finding that the few virus particles present in rectal swabs (10 to 60 TCD_{50}) were sufficient to paralyze monkeys inoculated intraspinally.

It is only these altered particles in the progeny of the vaccine virus which might become dangerous as they are passed about, under no control, in the community.

From the data presented it is evident that a definite increase in neurovirulence did take place in a substantial portion of the strains excreted by the vaccinated children in Mexico City. The effect of the spread of reverted strains in communities such as the one studied remains an open question

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DISCUSSION

DR FOX: I suppose the most obvious question stems from the implication and, in fact, the rather frequent statement that there has been a change in the character of these strains, when the obvious alternate interpretation here is that these feedings were done at a time when there was a considerable abundance of wild viruses, and that we have here a collection of both wild viruses and the progeny of the viruses that were fed, and that it is not easy to sort these out

DR SABIN. Dr Melnick in the past has carried out studies under much more favorable conditions, studies in the United States on material excreted by children in institutions, by children in homes, by adults under conditions where other viruses were not present, and she has published or unpublished data of great interest on some of these characters

The present study, in my opinion, was carried out under the most unfavorable conditions to try to determine precisely the word "change" I think the word "change" here is completely unwarranted, from Table 9 we have evidence that all three types of poliovirus were picked up without relation to feeding, and from Table 12 we have evidence in families, with perhaps one exception, where, whatever the sensitivity of the method of testing rectal swabs may be, in each case the index child was not excreting virus, and some contact was excreting virus

I think the conclusion is inescapable here, as Dr Fox has said, that you are dealing with circumstances in which you are coming in with something else when all types of wild viruses are spreading, which are of a different character, as we know

The second point is that in the past Dr Melnick has carried out certain studies on correlation of neurovirulence, which went beyond the million and ten million doses inoculated intracerebrally, and she has been able to show, certainly that viruses which tested as $d+$, even under more favorable conditions than these tests did not correlate with intracerebral virulence

In the present studies the tests on intracerebral inoculations in monkeys stopped at a million to ten million doses and did not go beyond, and therefore cannot be correlated with other tests on neurovirulence

I would like to make a point about the significance of the d character, as measured here in the closed bottles, or carried out by the original technique prescribed. It was not a question of failure of reproducibility, and it was not a question of too large a difference. The differences between the vaccine strain and the other strain were in the range of 100 000 and even with special mutants, they went down in progressive stages.

My conclusion from looking at these data is that there is sufficient irregularity in measuring the d character by this method to make it very dubious

Besides, as I pointed out yesterday, by careful back mutation studies it was possible to show that the d character bears no relationship to neurovirulence. The final point, it seems to me is that to attempt to study or speak of changes of genetic characters under conditions such as this, where all three types of wild poliovirus are spreading—as I showed also in our little study yesterday, where, even before starting, 3 out of 12 children had their own poliovirus—I think is almost impossible There is no denying the fact, which has been stated repeatedly, that alterations of neurovirulence are demonstrable by passages in the alimentary tract which are not progressively increased, and the tests carried out here are not of an order to permit comparison with anything that has been done heretofore

DR ABAD GOMEZ Dr Benyesh-Melnick showed some cases in which children of the families tested were excreting types of poliovirus before being fed the vaccine How does she know that the d and $d+$ that she was testing were not natural viruses occurring in nature?

DR BENYESH-MELNICK All the questions posed revolve around the problem of whether the strains isolated from the vaccinated children were progeny of the vaccine virus or whether [illegible]

of the polioviruses isolated from the vaccinees

we considered as homotypic all the viruses excreted after vaccine feeding and being of the same type as in the vaccine fed. Any poliovirus excreted beginning before the vaccine of that type was fed was considered as heterotypic wild virus. There were 15 such isolations, from 5 children, and they were excluded from the study of changes in neurovirulence. Thus all the tests on changes in monkey neurovirulence were performed with isolates considered to be a result of homotypic infection with the vaccine virus.

The correlation of homotypic infection after vaccine virus feeding is so high that it seems unlikely to us that the reverted viruses can be excused on the basis of being termed "wild viruses." This will be even more obvious when we present our second paper at the next session of this Conference.

Dr. Sabin: I think something should be pointed out here: that these are not favorable conditions under which to carry out such a study, that you have to make assumptions that there are these difficulties, and that therefore it would have been of great interest to see similar studies carried out under more favorable conditions, carried out by criteria comparable to those used before.

Dr. Melnick: We can only report the data as we obtained them in this study. I think that it is important to know what goes on in children fed vaccine virus other than in the United States, which is far cleaner in regard to the incidence of enterovirus infection than tropical areas.

Dr. Sabin raised the question earlier today as to the influence that bacterial flora might have on vaccine "takes" by the oral route. The flora of a child in Mexico City might very well be different from that of a child in Cincinnati. And this flora—as well as the enterovirus flora—might also influence *changes in the virus as it multiplies* in the gut. It seems essential that one should find out what goes on in this regard wherever the vaccine viruses are used.

If we were to consider all viruses with altered markers as "wild" viruses, and were to eliminate them from our results, then we would be left with the situation that the feeding of vaccine virus to the *Mexican children infected* relatively few of the group fed.

It should be recalled that we had 10 families in the study group as controls, and failed to isolate any wild polioviruses from them during the 10 weeks of the field study. The incidence of excretion of other enteroviruses in the control families was the same as that in the families fed the vaccine.

Let us look again at the child selected as the donor of the virus tested in the chimpanzee. Intentionally, we selected an infant that would seem to have had less contact with other children. This was a 2-month-old baby, for as Dr. Sabin pointed out yesterday, the chances of young infants becoming infected from contacts with others are less than is the case with older children.

This child, after getting Type 1 vaccine, excreted Type 1; and after getting Type 3 vaccine, excreted Type 3. Now we are being asked to regard this pattern of excretion as having nothing to do with the vaccine feeding.

Dr. Ramos Alvarez carried out antibody studies on these vaccinated children, and their antibody development correlated to a high degree with the virus type he fed and the virus type we isolated.

Dr. Sabin said that he was very dubious about our method of performing the tests for the *d* marker. Had he paid more attention to the data on the slides, he would have noted the reproduci*bility of the results. As pointed out in our pres*entation, attenuated LSc and virulent Mahoney were included in each run with the strains under test, and it was always in relation to the results of the two controls that the data were evaluated.

It should be recalled that a virus with the $d-$ marker will still grow at a low bicarbonate concentration. The letter "d" stands for "delay." In Dulbecco's original work on this marker he showed that in plates in a CO_2 incubator, the number of plaques at low bicarbonate tend to equal those obtained at high bicarbonate if the plates are observed for a few additional days. Our findings in bottles have agreed with Dulbecco's in plates insofar as being able to identify $d-$ and $d+$ strains is concerned.

On the one hand, Dr. Sabin is arguing that our data are not meaningful because he believes that the isolates represent wild viruses rather than reverted vaccine viruses, and on the other that the viruses have not reverted because our methods for showing changes are inadequate. I do not see how he can have it both ways, for he appears to be arguing in opposite directions.

Dr Sabin referred to Table 10 as indicating that wild polioviruses were present in the area This table was meant to show that very point. In it are listed the data on all of the 5 children infected with heterotypic viruses We feel that it is important to know what goes on when oral vaccine is used in an area where some children are infected with wild viruses However, it does not follow that every isolation associated with increased neurovirulence can be explained away as due to wild viruses If wild viruses were that prevalent, there would not be any need to use a vaccine

DR KOPROWSKI: Dr Melnick has presented results of his intraspinal inoculation of two chimpanzees; one was injected with the vaccine virus, and the other with the same virus after one passage through human intestinal tract. One of the chimpanzees became paralyzed, and the other not I believe it is rather risky to draw any conclusion from such a type of experiment.

Dr Courtois and I have injected 39 chimpanzees by the intraspinal route with different preparations and different variants of attenuated strains Four chimpanzees out of 39 became paralyzed, and 10 out of 39 had lesions of CNS. One of the strains used, the old SM virus, had a $d+$ character, and out of 5 chimpanzees injected intraspinally, one was paralyzed with specific CNS lesions These results prompt me to warn Dr Melnick to use caution in his interpretation of his data obtained in two chimpanzees

DR MELNICK If one selects random samples for the very expensive neurovirulence test in chimpanzees, then one might very well select unmodifid virus progeny Certainly all the virus progeny are not changed after multiplication in the alimentary tract

We purposely selected a strain for which the *in vitro* tests had shown reversion from the $d-T-$ markers of the vaccine to the $d+T+$ markers characteristic of virulent viruses If Dr Courtois had not screened his strains for changes before testing in chimpanzees, then I am not surprised at the results which he obtained I presume that all chimpanzees showed evidence that the inoculum had been placed properly in the spinal cord

The issue here is whether the virus exhibited a quantitative change. Dr. Sabin has reported on a number of chimpanzees in which his vaccine strains failed to multiply in the spinal cord. These strains seem to be truly avirulent for the chimpanzee.

True, one animal constitutes a small test, but the extent and progression of the paralysis and the confirmatory histological lesions prove that the vaccine strain after human passage contains chimpanzee neurovirulent virus

DR SABIN: For the record, I want to say that Dr. Melnick told me that the one chimpanzee he did inoculate was inoculated with a dose of about a million or more. Is that correct?

DR. MELNICK. About one million tissue culture doses were inoculated.

DR SABIN: And also inoculated by a procedure that was somewhat different

The other point for the record is that among the children who were vaccinated and who did not excrete enough virus to be detected by rectal swab that we discussed in Table 12, you mentioned serologic data of Dr Ramos Alvarez I had an opportunity to go over the table and the data on a number of these children with Dr Ramos Alvarez Some of those who did not excrete virus after feeding actually had antibody, naturally acquired antibody for that type, and would not have been expected to excrete. Therefore, the appearance of that type of virus in the contact, subsequently, is much more likely to be explained on the basis that those children playing around with others have picked it up from them, rather than having picked it up from the vaccinated child

DR BENYESH-MELNICK Dr Sabin is right in that some of the children had prevaccinal antibodies, but certainly not all of them This question will be answered in detail in the paper to be presented this afternoon

DR SABIN Under Family C, child age fourteen months fed Type 1 had, prior to feeding, antibody for Types 2 and 3, and Type 3 virus appeared in a contact at a time before it appeared in the vaccinee.

The same for Family G. They had a child four months of age who did not excrete the Type

1 that was fed, but a contact three years old did excrete Type 1 virus.

Dr. Bodian: I think we are talking around the main issue posed by Dr Benyesh-Melnick's beautiful presentation The main issue touches on what is probably the most essential problem in this entire field, and I think we ought to talk about this. There is no time now, of course, but it is going to be necessary later in this Conference to examine very carefully the implications of her results, which indicate the successive isolations of a specific virus type after virus feeding These isolations repeatedly go from *d* character to *d*+, and there is a correlation of the *d*+ character with neurovirulence

It seems to me that, irrespective of all the things that have been said about details which may be at fault, about methods which may not be 100 per cent consistent, we will have to address ourselves to the important aspect of her presentation, which is the possibility that we are dealing with reversion to neurovirulence.

Chairman Stuart-Harris: Any other questions? Do you wish to postpone the issue until later this morning?

Dr Benyesh-Melnick Yes

Dr Melnick Dr Sabin's question will be answered in detail during this afternoon's session when we shall discuss the antibody responses of the children in this study

2. REVISED PRELIMINARY REPORT ON THE LOUISIANA OBSERVATIONS OF THE NATURAL SPREAD WITHIN FAMILIES OF LIVING VACCINE STRAINS OF POLIOVIRUS *

HENRY M. GELFAND, M.D., LOUIS POTASH, PH.D., DOROTHY R. LEBLANC, R.N., AND JOHN P. FOX, M.D., PH.D.

Division of Epidemiology, Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, Louisiana

DR. GELFAND (*presenting the paper*): With the isolation, selection, purification and large-scale production of attenuated variant strains of polioviruses,[1] we are offered the opportunity to substitute live-virus vaccination against poliomyelitis for the formalin-inactivated vaccine in current use. There are many impressive and generally accepted advantages of vaccination with these infectious strains, and a few technical disadvantages that may be overcome in the future.

Since live-virus vaccination results in active intestinal infection, the spread of infection to persons in intimate association with the vaccinee was to be anticipated, and several investigators, using various strains of all 3 types, have demonstrated spread to susceptible contacts under various conditions of exposure.[2] The occurrence of this phenomenon probably would be, in itself, of little concern were it not for the possibility that the fecally excreted viruses being spread may have undergone back-mutation to a level of neurovirulence greater than was acceptable in the original oral vaccine. One might well take an even more extreme position—that, were it not for this possibility, dissemination of vaccine viruses by contagion is a decided advantage of live-virus vaccination in that the administration of vaccine to only a portion of the population might result in the effective immunization of the entire group. This would be vaccine-induced "herd protection" of a level not yet achieved with any other immunizing agent.

Whether vaccine virus spread is to be applauded as an advantage or feared as a danger, knowledge of the infectiousness and transmissibility of the vaccine strains is essential to their rational use in field trials, and eventually, in routine practice. This communication reports results of studies on the capacity of the set of attenuated polioviruses comprising the "Sabin vaccine" to spread within families living under normal conditions in southern Louisiana. A preliminary report of this study is to be published soon in the Journal of the American Medical Association,[3] and the data to follow constitute a current revision of the still continuing laboratory investigation, including changes resulting from both the re-examination of specimens and the completion of additional household units.

MATERIALS AND METHODS

The Population Studied

Families were recruited for the present investigation in 1958 from among a group in New Orleans and Baton Rouge which had been under our observation for over four years in a study of natural infection with polioviruses.[4] Their infection history was therefore unusually well known. During 1956 and 1957 all persons above the age of 6 months and not naturally immune to all 3 types had been given 3 suitably spaced inoculations with "Salk vaccine."

To be eligible for the present study, a family had to include, for any given type, at least 3 "natural-susceptible" members, defined as persons without prior natural homologous infection as observed by us and coupled with the complete absence of *naturally acquired* serum neutralizing antibody. On this basis, a family might be "eligible" for more than one type, and many were used 2 or 3 times. Each feeding and family observation was therefore designated as a separate "unit." In addition in sev-

* Aided by a grant from The National Foundation.

1 that was fed, but a contact three years old did excrete Type 1 virus

Dr Bodian I think we are talking around the main issue posed by Dr Benyesh-Melnick's beautiful presentation. The main issue touches on what is probably the most essential problem in this entire field, and I think we ought to talk about this There is no time now, of course, but it is going to be necessary later in this Conference to examine very carefully the implications of her results, which indicate the successive isolations of a specific virus type after virus feeding These isolations repeatedly go from *d* character to *d*+, and there is a correlation of the *d*+ character with neurovirulence

It seems to me that, irrespective of all the things that have been said about details which may be at fault, about methods which may not be 100 per cent consistent, we will have to address ourselves to the important aspect of her presentation, which is the possibility that we are dealing with reversion to neurovirulence

Chairman Stuart-Harris: Any other questions? Do you wish to postpone the issue until later this morning?

Dr. Benyesh-Melnick: Yes

Dr Melnick Dr Sabin's question will be answered in detail during this afternoon's session, when we shall discuss the antibody responses of the children in this study.

TABLE 1. SCHEDULE OF SPECIMEN COLLECTION

Donors	Type of Specimen	Days after Vaccine Feeding on which Indicated Specimens were Collected		
		Complete Schedule	Modified Schedule	Abbreviated Schedule
Index	Blood	0, 3, 7, 11, 14, 18, 21, 60	0, 8, 20, 60	0, 60
	Pharyngeal	0–14	0, 2, 4, 8	None
	Fecal	0–14, 18, 21, 25, 28, 1x per week to termination	0, 2, 4, 8, 1x per week to termination	0, 1–2x per week for variable period
Family contacts	Blood	0, 60	0, 20, 60	Same as index
	Pharyngeal	3–17	0	
	Fecal	0, 3–17, 21, 25, 28, 31, 1x per week to termination	Same as index	
Extra-household contacts	Blood	0, 60	None	None
	Fecal	7, 14, 21		
Number of household units completed by indicated schedule		26	17	24 (plus 8 immune feedings)

tion, this atypical result suggests that he was, in fact, naturally immune There is no other suggestion that the age of the fed person affected the likelihood of infection

Among the 61 persons who did become infected, fecal excretion was the most sensitive indicator of the fact Pharyngeal excretion was usually detected following the administration of the 7 log doses (20 of 30 persons), but was found much less often after the 5 to 6 log doses (5 of 23 persons) The serologic detection of *infection was even less reliable Study of appropriate specimens of all persons is not yet complete, but as indicated in Figure 1 (which includes the results of infected susceptible contacts as well), significant rises above the titers resulting from "Salk" vaccination often did not occur with the higher pre-infection titers In no instance did a significant serologic titer rise occur in any person who *failed* to excrete virus

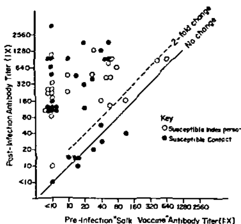


FIG 1 Homologous antibody titers before and after proved *infection with "Sabin vaccine"* in natural-susceptible persons

eral instances the same virus type was fed more than once in a single household when the first feeding failed to cause infection and/or spread. On a few occasions, the vaccine was purposefully fed to naturally immune persons in households which also contained 2 homologously susceptible persons as indicators of spread. Family units were assigned to one of 3 socioeconomic categories: White-upper economic, White-lower economic, and Negro. The economic division was rather well correlated with social and sanitary facilities and practices such as household crowding, adequacy of toilet and hand-washing equipment, and "personal hygiene." Negro and White-lower economic units usually are combined below, and referred to as "lower economic."

Virus Strains and Vaccine Administration

The viruses used were kindly provided by Dr. Albert Sabin, and were from the single large pools of each type undergoing extensive field trials at the present time. The stock material, diluted to contain the desired dose, was distributed in 1.2 ml. aliquots into individual screw-topped vials, and kept frozen until used. Repeated titration of sample vials showed that an average of 5.3, 5.8, and 7.3 logs (Type 1) and 5.1, 5.6 and 7.1 logs (Types 2 and 3) had been used. A few persons received 3.6 log doses early in the study.*

Administration was done in the home by one of the authors (D. R. L.) by squirting 1 ml. of the suspension into the back of the mouth of the recipient, followed by drinking a glass of water. Individual "medicine droppers" were used in administration, and extreme care was taken to prevent contamination of the skin or of objects in the home.

Method of Study and Collection of Specimens

For each family unit, one susceptible individual was fed and designated the "index" person and the other susceptible and the naturally immune household members were all observed as "family contacts." When observations were made on spread to others not living in the same domicile, such persons are referred to as "extra household" (EHH) contacts.

The various schedules of specimen collection are outlined in Table 1. Day "0" is the day of feeding in all instances. These schedules were guides only, and often had to be modified.

Blood specimens were almost always collected by venipuncture; pharyngeal specimens were obtained from adults and older children by gargling, and from small children by throat swabbing. Fecal specimens consisted of a pellet of whole feces.

During the investigation of a number of family units, an attempt was made to detect the presence of poliovirus on the skin of some children and on the floor, on toys, etc., in some homes. For this purpose, wet cotton pledgets were swabbed over the appropriate surface, and were returned to the laboratory where they were squeezed almost dry.

Laboratory Procedures

Rhesus monkey kidney-cell-tissue cultures, locally prepared in our laboratory, were employed throughout, using standard and acceptable techniques for virus isolation and the direct cytopathic test for antibody determinations.[8]

RESULTS

Data relating to 74 family units are the subject of this report. In 66, a natural-susceptible person was fed, the distribution by socio-economic and family status being: Negro—17 children, White-lower economic—14 children, and White-upper economic—24 children and 11 parents. In 8 additional units, a homologously natural immune was the vaccine recipient.

Infections in Susceptible "Index" Persons

Of these 66 feedings, all but 5 resulted in the infection of the index person as indicated by the subsequent recovery of homologous virus from the pharyngeal and/or fecal excretions. As may be seen in Table 2, 4 of these failures were in persons who had received the small dose of Type 1 vaccine. One of these was an adult. He was refed with 5.3 logs of the same virus type, and again failed to become infected. He was refed once more, with 7.3 logs, and thereupon excreted fecally for 5 days. Despite the previous absence of neutralizing antibody in this man, demonstrated during 4 years of observa-

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* In a recent communication,[2f] Sabin reports that higher titers of vaccine pools may be obtained by titration in cynomolgus kidney cell tissue cultures with an improved technique. Based on his reports, the doses we used may have been somewhat greater than reported here.

TABLE 3 DURATION OF PHARYNGEAL AND FECAL EXCRETION OF POLIOVIRUSES FOLLOWING ORAL ADMINISTRATION OF "SABIN VACCINE"

Observed Duration of Excretion (Days)	Numbers of Persons With Indicated Duration Following Ingestion of Indicated Virus Type					
	Type 1		Type 2		Type 3	
	Pharyngeal	Fecal	Pharyngeal	Fecal	Pharyngeal	Fecal
1	1	0	0	0	0	0
2	0	1	1	0	0	0
3	0	0	1	0	1	0
4	1	0	0	0	0	1
5	1	1	2	1	0	0
6–10	7	6	3	6	3	1
11–15	1	6	0	1	2	2
16–20	1	4	0	1	0	2
21–30	0	3	0	3	0	3
31–40	0	2	0	4	0	5
41+	0	1	0	1	0	6
Mean duration	7.4	20.5	5.0	20.6	9.2	38.6
Mean interval*	1.8	4.5	1.4	4.6	1.3	3.5

* Average time elapsed between the last negative specimen before excretion began and between the last positive and the first negative specimen after excretion ended.

interval between ingestion of vaccine and onset of excretion, was very short in the majority of index persons on whom daily observations were made. As shown in Table 4, almost all such persons had virus in the pharyngeal secretions by the second day after feeding, and over 60 per cent demonstrated fecal virus by the first day. Virus type and dose appeared to have had no effect.

The results of antibody titrations in those infected index persons from whom repeated blood specimens were collected at frequent intervals are presented in Table 5. The rise in titer took place between the seventh and tenth day in the majority, and there is a suggestion that the titer had already begun to decline within 2 months after infection.

In addition to the repeated feeding of the adult already mentioned, one other susceptible index person is worth noting in detail. Following the administration of 3.6 logs of Type 3 to

TABLE 4 INTERVAL BETWEEN INGESTION OF "SABIN VACCINE" AND ONSET OF DETECTABLE EXCRETION OF HOMOLOGOUS VIRUS, IN NATURAL-SUSCEPTIBLE PERSONS FROM WHOM DAILY SPECIMENS WERE COLLECTED

Interval Between Ingestion and Excretion (days)	Number of Persons with Virus Detected at Indicated Site After Indicated Interval	
	Pharynx	Feces
1	3	25
2	6	14
3	1	1
4	0	0
5	1	1
All	11	41

TABLE 2 SUMMARY OF EFFORTS TO RECOVER VIRUS FROM PHARYNGES AND FECES OF INDEX PERSONS FED "SABIN VACCINE", BY VIRUS TYPE, DOSE AND FAMILY STATUS

Vaccine Virus Type	Dose Fed (Logs)	Family Status	Homologous Virus Detected in Appropriately Collected Specimens: Pharyngeal, No. Persons Examined	Pharyngeal, No. Persons Positive	Fecal, No. Persons Examined	Fecal, No. Persons Positive
1	3.6	Child (2–8 yrs.)	3	0	3	0
		Adult	1	0	1	0
	5.3	Child (2–8 yrs.)	4	1	6	6
		Adult	2	0	2	1
	5.8	Child (3–6 yrs.)	4	2	4	4
		Adult	0	–	0	–
	7.3	Child (2–5 yrs.)	8	6	8	8
		Adult	4	3	5	5
2	5.1	Child (2–6 yrs.)	5	0	6	6
		Adult	0	–	0	–
	5.6	Child (4–6 yrs.)	2	2	2	2
		Adult	0	–	0	–
	7.1	Child (2–6 yrs.)	7	4	8	8
		Adult	1	1	1	1
3	3.6	Child (1–2 yrs.)	2	0	2	2
		Adult	0	–	0	–
	5.1	Child (3–7 yrs.)	5	0	6	6
		Adult	0	–	0	–
	5.6	Child (4 yrs.)	1	0	1	1
		Adult	0	–	0	–
	7.1	Child (2–6 yrs.)	8	5	9	9
		Adult	2	1	2	2

The duration of excretion in index persons (Table 3) was very variable, in the pharynx from 1 to 17 days and in the feces from 2 to at least 137 days. There appeared to be no relationship between duration *and either* the size of *the* infecting dose or the age of the recipient. It appears that excretion of Type 3 virus may last longer than that of other types on the average, although the longest duration in this series, 137 days with no endpoint being reached, was with Type 1.

The incubation period, as measured by the

Table 6 Homologous Infections Occurring Among Family Contacts of Index Persons Infected Following Oral Administration of "Sabin Vaccine"

Natural Immunity Status of the Family Contacts	Family Status of the Index Person	Virus Type	Contacts in Upper Economic Families				Contacts in Lower Economic Families			
			Occurrence of Pharyngeal Virus Excretion in Index Person				Occurrence of Pharyngeal Virus Excretion in Index Person			
			Yes		No		Yes		No	
			No of "Units"	Ratio* of Contact Infections	No of "Units"	Ratio* of Contact Infections	No of "Units"	Ratio* of Contact Infections	No of "Units"	Ratio* of Contact Infections
Susceptible	Child	1	5	2/11	6	0/18	4	3/13	3	0/9
		2	2	0/4	2	0/5	4	9/11	8	5/25
		3	1	0/2	6	3/23	4	12/12	7	10/19
	Parent	1	3	0/10	2	0/6	-	—	-	—
		2	1	0/3	—	—		—	-	—
		3	1	0/3	1	0/4	-	—	-	—
Immune	Child	1	5	0/9	6	0/17	4	0/10	3	1/8
		2	2	0/5	2	0/2	4	0/18	8	2/21
		3	1	0/3	0	0/7	4	9/15	7	9/33
	Parent	1	3	0/2	1	0/1		—		—
		2	1	0/4		—	-	—		—
		3	1	0/1	1	0/1	-	—		—

* Ratio of persons infected (numerator) to family contacts present in the home (denominator).

TABLE 5 ANTIBODY RESPONSE OF INFECTED NATURAL-SUSCEPTIBLE INDEX PERSONS

VIRUS TYPE	DOSE FED (LOGS)	AGE OF INDEX PERSON	ANTIBODY TITER ON INDICATED DAY FOLLOWING INGESTION							
			0	3	7	10	14	17	20	60
1	5 3	4	10	<10	56	900	900	800	1000	450
	5 3	F	32	16	20	250	400	450	510	450
	7 3	M	28	50	50	510	900	640	1000	510
2	5 1	2	80	50	56	510	510	1000	1280	640
	5 1	3	50	80	56	400	400	400	640	400
	7 1	3	450	640	320	450	1280	900	1800	800
	7 1	5	2	4	10	640	900	1000	800	320
	7 1	5	125	110	110	510	510	510	640	220
	7 1	M	32	32	14	110	200	220	160	125
3	3 6	1	—	64	56	—	56	56	56	—
	3 6	2	<10	<10	<10	40	32	56	50	250
	5 1	5	<2	<10	<10	32	32	160	400	220
	5 1	7	56	56	56	510	510	900	1000	450
	7 1	2	<2	<2	<2	28	57	160	160	80
	7 1	4	<2	<2	6	220	220	400	510	110
	7 1	5	<2	<2	<2	14	220	—	320	1000

a little girl, she had 5 days of fecal excretion but no serologic response She was refed with 5.1 logs of the same type and thereafter excreted for 29 days and experienced a marked rise in antibody titer The first infection in this child may represent the multiplication of virus in so limited an area of the intestine that full evolution of the infection had not occurred, the antigenic stimulus was weak, and she remained fully susceptible, acting as a non-immune upon re exposure.

Infection Attempts in Natural-immune Persons

Eight natural-immunes have so far been studied following ingestion of "Sabin vaccine", 4 with Type 1, and 2 each with Types 2 and 3 All were children and all were fed the larger doses. Six failed to become reinfected. One each excreted Type 1 and Type 2 in the feces only for 9 days

Spread of Infection from the Index Person

Because of the large number of variables, any or all of which may have important influences on the spread of vaccine viruses within households, the combination of units with identifiable differences is not fully justified Table 6, therefore, reports the ratio of infections among natural-susceptible and natural-immune family contacts, within the few units included in each category Since the dose fed seemed to have little effect on the infected index person other than in determining the likelihood that pharyngeal excretion would occur, only the presence or absence of the latter is included in the table The determination of contact infection was in all cases based on the occurrence of fecal excretion since only once was pharyngeal virus detected in a contact, and a rise in antibody titer never occurred without the detection of excretion

Examination of this table shows a number of interesting contrasts Among the susceptible family contacts of infected index children, many infections occurred, but there was a marked difference based on economic status, the lower economic families experiencing many more infections Among the 3 virus types, Type 3 feedings appeared to have resulted in more spread The presence of pharyngeal excretion may have been associated with somewhat more extensive

TABLE 8 SAMPLE FAMILIES TO ILLUSTRATE SPREAD OF "SABIN VACCINE" AFTER ADMINISTRATION TO A SINGLE NATURAL SUSCEPTIBLE INDEX CHILD (THE LATTER INDICATED BY ARROW)

Age (Years)	Homologous Natural Immunity Status	Homologous Antibody Titer 1 x on Day 0	Homologous Antibody Titer 1 x on Day 62	Presence of Homologous Virus in Feces on Day— 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
a Unit #6 Negro family, index fed 5 3 logs of type 1 virus																		
F	Immune	640	320	§−	−	−		−		−		+	+		−			−
M	Immune	80	160	−		−		−				−	−	−				−
12	Susceptible	28	20	−			−		−		−	−		−			−	−
9	Susceptible	28	20	−	−		−		−	−				−			−	
8	Susceptible	10	14	−		−	−		−			−	+			−		−
7	Susceptible	20	320	−	−			−		+		+		+		+		
5	Susceptible	<2	10	−	−		−			+	−		−			−		−
→3	Susceptible	10	220	−	−*		+			+	+		+				+	
1	Susceptible	<2	640	−		−	−		−	−			+			+		+
b Unit #39 White-lower economic family, index fed 5 1 logs of type 3 virus																		
F	Immune	20	14	−	−						−			+				
M	Immune	28	20	−	−				−		−			−				
11	Immune	450	640	−		−			−		+		−			+	−	
9	Immune	160	160	−		−	−		−		+	−		+				+
6	Susceptible	5	110	−	−		−			+		+					+	
5	Susceptible	<2	110	−	−	+			−	+	+	−				+		
→3	Susceptible	<2	160	−	+*	+			+	+			+			+		
2	Susceptible	<2	110	−	+				−		+		+	+			+†	

* No virus isolated from pharyngeal specimens collected on day 1 through 10
† Homologous poliovirus isolated from kitchen floor
§ Indicates specimen tested and found negative, blank space indicates no specimens available

TABLE 7 SUMMARY OF HOMOLOGOUS INFECTIONS OCCURRING AMONG FAMILY CONTACTS OF INFECTED INDEX CHILDREN, BY ECONOMIC STATUS AND VIRUS TYPE

VIRUS TYPE	UPPER ECONOMIC FAMILIES		LOWER ECONOMIC FAMILIES	
	RATIO* OF CONTACT INFECTIONS	PERCENTAGE OF CONTACT INFECTIONS	RATIO* OF CONTACT INFECTIONS	PERCENTAGE OF CONTACT INFECTIONS
	a Susceptible contacts			
1	2/29	7	9/22	41
2	0/9	0	14/36	39
3	3/25	12	22/31	71
All	5/63	8	45/89	51
	b Immune contacts			
1	0/26	0	1/18	6
2	0/7	0	8/39	21
3	0/10	0	18/48	38
All	0/43	0	27/105	26

* As in Table 6

spread than occurred in its absence, although a number of these contact infections apparently took place after pharyngeal excretion had ceased Despite the fact that examples were few in number and restricted to the upper economic group, it is striking that no single instance of spread followed the infection of an index adult Finally, the rather large number of reinfections among lower economic natural-immunes is notable

Differences based on economic status and virus type among the contacts of index children are summarized in Table 7. The "benefit" of lower economic status and the generally greater infectiousness of Type 3 vaccine are now more apparent.

No family contact infections resulted from the successful feedings in natural immunes with Types 1 and 2

The interval between onsets of excretion in the index person and his contacts appeared to be very variable. On several occasions, it was as short as one day, and on others not less than 1, 2 or 3 weeks. Viral excretion by natural immunes was often greatly delayed The infection records of 2 typical lower economic families are reproduced in Table 8

Studies of EHH contacts were initiated where conditions for spread were likely to be optimal, i e with the administration of 7 log doses in lower economic families, and an adequate record was made in connection with 4 study units (Table 9)

In Unit 31, neither family nor EHH contacts became infected despite prolonged fecal excretion by the index child and frequent, intimate contact.

Unit 45 is remarkable in that a single 2-hour period of contact with only the infected index child resulted in the infection of 2 out of 3 child visitors In Unit 49 also there was intimate play contact for only 2 hours, but with all 4 infected children, and only 1 out of 3 child visitors became infected The 2 who missed in-

TABLE 10 DURATION OF FECAL EXCRETION OF HOMOLOGOUS POLIOVIRUSES BY FAMILY CONTACTS OF PERSONS FED "SABIN VACCINE"

OBSERVED DURATION OF EXCRETION (DAYS)	NUMBERS OF FAMILY CONTACT PERSONS					
	IMMUNE			SUSCEPTIBLE		
	TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3
1	4	7	10	5	0	3
2	1	1	2	0	1	0
3	0	1	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6–10	0	1	2	0	2	4
11–15	0	0	1	1	4	3
16–20	0	0	1	0	1	6
21–30	0	0	1	2	1	1
31–40	0	0	1	2	3	2
41+	0	0	0	1	2	6
Mean duration	1 2	2 1	6 6	17 5	25 7	24 9
Mean interval*	6 6	2 1	4 3	4 1	4 3	7 8

* As in Table 3

fection had a similar opportunity 2 weeks later and again escaped

An entire study was planned around Unit 46. Agreement to participate had been obtained from a long series of related families who agreed to regulate contact amongst their children in serial fashion on a schedule to be determined by us Unfortunately, the second family-passage failed It is perhaps significant that at the time of contact between families, the index household and this contact household were *undergoing natural episodes with ECHO* virus Type 1 and polio 2, during which 4 of the 5 susceptible contact children were infected with one or the other or both "wild" viruses However, 3 out of 4 other EHH contacts in 3 households did contract infection with Type 3

Nature of the Infections Experienced by Contacts

Only on one occasion was homologous virus isolated from a pharyngeal specimen *from a* contact The durations of fecal excretion are indicated in Table 10 The significance of the shorter mean duration for Type 1 excretion is doubtful Of greater importance is the rather large number who excreted for very short periods of time, and who, therefore, may not have had an effective immunizing experience. It is probably also unlikely that they can be instrumental in further virus spread This is particularly characteristic of immunes, but it is also true of a substantial proportion of the susceptible contacts Since *all* of the persons who excreted for only one day also failed to experience a booster rise in neutralizing titer, and since in many instances fecal specimens were not obtained daily from contacts, it is quite likely that a number of other brief contact infections were missed Many of the infected contacts did, however, experience extended periods of excretion

Serologically, the frequent absence of a rise from *low* initial antibody titer in infected contacts as compared with index persons is notable *(Figure 1) and is related to the frequency of* transient infections in the contacts. None of the re-infected immune individuals studied experienced a rise in titer

TABLE 9. SUMMARY RECORD OF HOMOLOGOUS INFECTIONS AMONG EXTRA HOUSEHOLD (EHH) CONTACTS OF FAMILIES IN WHICH ONE MEMBER WAS FED "SABIN VACCINE"

Unit No	Index Child						Susceptible Child Family Contacts		Susceptible Child EHH Contacts		Notes
	Virus Fed		So-Ec Status*	Age (Yrs)	Virus Excretion at Time of Contact		Number	Number Infected	Number	Number Infected	
	Type	Dose			Pharyngeal	Fecal					
31	2	7 1	N	3	No	Yes	2	0	1	0	Frequent, intimate contact
45	3	7 1	W-L	4	Yes	Yes	4	4	3	2	Contact with index only, 2 hours on Day 6
40	3	7 1	N	3	Yes	Yes	3	3	3	1	Intimate contact with all children for 2 hours on Day 5
					No	Yes	–	–	2	0	Intimate contact with all children for 2 hours on Day 19
46	3	7 1	W-L	4	Yes	Yes	4	4	5	0	EHH family had concurrent infection with ECHO 1 and Polio 2
							–	–	2	1	EHH family had concurrent infection with ECHO 1
							–	–	1	1	All 4 EHH families had contact only with siblings of index child of Unit # 16 and with each other
							–	–	1	1	

* N=Negro; W L=white, lower economic

Coincidental "Wild" Enteroviral Infections

Several episodes of infection with other enteric viruses occurred coincidentally with the administration of the "Sabin vaccine", including: poliovirus Type 2, ECHO virus types 1, 3, 6, 9, and 11, Coxsackie virus Types A9 and B5, and unidentified adenoviruses On several occasions, interference between these agents and the attenuated strains was strongly suggested Table 11 gives 3 examples In Unit 10, Coxsackie B5 was infecting all 3 susceptible children at the time that Type 1 poliovirus was fed to the 5-year old The large dose administered may have suppressed further multiplication of the C-virus in the index child but the smaller dose received by the others, by way of contamination, appears not to have been sufficient, and they escaped Unit 15 may illustrate reciprocal interference between a "wild" Type 2 and the attenuated Type 1 At a later time, after both types had disappeared from the family, the index child became infected with Type 2, perhaps from a neighborhood source outside the home In Unit 46, an ECHO type 1 episode was either just beginning or nearing termination in the household when the index child was fed with attenuated Type 3 The ECHO infections spontaneously disappeared or were suppressed by the spreading poliovirus, and eventually all susceptible children became infected In addition to other episodes similar to the above many casual infections with enteroviruses were detected, including an ECHO 9 isolation from a pharyngeal gargle, later during the course of unit investigations when interference could not be postulated

Virus Recovery from the Skin and from Fomites in Households

During the course of the investigation of 26 study units, some attempt was made to detect the presence of homologous poliovirus from external sites This was not carefully organized but was made in those households with the poorest hygienic standards The places examined by swabbing included most commonly the skin of the hands, feet, and buttocks (not perianal) of an index child or his infected siblings, toys, floors, and doorknobs In 5 units, an enteric virus was recovered as shown in Table 12. Note that in one household the virus recovered was of a type not fed as vaccine but which was concurrently present as a "wild" invader

Illness Associated with Infection with Attenuated Vaccine Viruses

Despite intensive and leading questioning, no evidence could be found to suggest that any illness, however minor, could be related to the infections produced by the administration or spread of the vaccine virus strains.

DISCUSSION

Although our primary objective was the study of the transmission of infection, the results relative to primary infection in the vaccine recipient are of interest With oral inocula consisting of

TABLE 12 RECOVERY OF ENTERIC VIRUSES FROM EXTERNAL SITES IN HOUSEHOLDS UNDERGOING EPISODES OF INFECTION WITH ATTENUATED "SABIN VACCINE"

Unit Number	So-Ec Status*	Type Fed	Number of Susceptibles Infected	Viruses Recovered	
				Type	Site
28	N	P2	3	P2	Buttock of index child
39	W-L	P3	4	P3	Kitchen floor
40	N	P3	3	P3	Buttock of sibling
49	N	P3	4	P3	Hands (4 occasions) and buttock of index child
15	N	P1	1 (plus 3 with P2)	P2	Living room floor

* As in Table 9

TABLE 11 EXAMPLES OF FAMILIES EXPERIENCING EPISODES OF NATURAL "WILD" ENTEROVIRUS INFECTIONS COINCIDENT WITH THE ADMINISTRATION OF "SABIN VACCINE"
(fed "index" child indicated by arrow)

a Unit #10: Upper economic, index fed 5.8 logs type 1

Age (Years)	Natural Immunity Status*	Presence of Virus in Feces on Day— 0	1	2	4	7	9	14	21
F	Immune	–§		–	–	–		–	–
M	Immune	–		B5	B5	B5	B5		B5
8	Susceptible	B5	B5		B5	B5	B5	B5	
→ 5	Susceptible	B5	B5	1	1	1	1		1
2	Susceptible	B5		B5	B5		B5	B5	B5

b Unit #15: Negro, index fed 7.3 logs type 1

Age (Years)	Natural Immunity Status*	0	2	3	6	12	20	38	55
F	*Immune*	–		–	–				
M	Immune	–		–	2	–	–	–	
6	*Susceptible*	2		2	2	–	–	–	
5	Susceptible	–		2	2	2	–	–	
4	Susceptible	2		2	2	2	–	–	
→ 2	Susceptible	–	1	1	1	1	1	–	2

c Unit #46: White-lower economic, index fed 7.1 logs type 3

Age (Years)	Natural Immunity Status*	0	2	4	5	7	8	10	26
F	Immune	–	–	–	–	–	–	–	
M	Immune	–	–	–	3	–	–	–	
18	Immune	–	–	–	–	–		–	
13	Immune	–	–	–		–	–	–	
12	Susceptible	–	–	–		3		–	3
10	Susceptible	–		E1+3		–	3	–	3
8	Susceptible	E1	E1	E1	3	E1	3	–	3
5	Susceptible	–		E1	–	–	E1	3	3
→ 4	Susceptible	–	3		3		3		3
1	Susceptible	E1	E1					3	3

* With reference to vaccine virus type
§ As in Table 8

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UNIT NUMBER	SO-EC STATUS*	TYPE FED	NUMBER OF SUSCEPTIBLES INFECTED	VIRUSES RECOVERED	
				TYPE	SITE
28	N	P2	3	P2	Buttock of index child
39	W-L	P3	4	P3	Kitchen floor
40	N	P3	3	P3	Buttock of sibling
49	N	P3	4	P3	Hands (4 occasions) and buttock of index child
15	N	P1	1 (plus 3 with P2)	P2	Living room floor

* As in Table 9

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(fed "index" child indicated by arrow)

AGE (YEARS)	NATURAL IMMUNITY STATUS*	PRESENCE OF VIRUS IN FECES ON DAY—							
a Unit #10 Upper economic, index fed 5 8 logs type 1									
		0	1	2	4	7	9	14	21
F	Immune	–§		–	–	–		–	–
M	Immune	–		B5	B5	B5	B5		B5
8	Susceptible	B5	B5		B5	B5	B5	B5	
→ 5	Susceptible	B5	B5	1	1	1	1		1
2	Susceptible	B5		B5	B5		B5	B5	B5
b Unit #15 Negro, index fed 7 3 logs type 1									
		0	2	3	6	12	20	38	55
F	Immune	–		–	–				
M	Immune	–		–	2	–	–	–	
6	Susceptible	2		2	2	–	–	–	
5	Susceptible	–		2	2	2	–	–	
4	Susceptible	2		2	2	2	–	–	
→ 2	Susceptible	–	1	1	1	1	1	–	2
c Unit #46 White-lower economic, index fed 7 1 logs type 3									
		0	2	4	5	7	8	10	26
F	Immune	–	–	–	–	–	–	–	
M	Immune	–	–	–	3	–	–	–	
18	Immune	–	–	–	–	–		–	
13	Immune	–	–	–		–	–	–	
12	Susceptible	–	–	–		3		–	3
10	Susceptible	–		E1+3		–	3	–	3
8	Susceptible	E1	E1	E1	3	E1	3	–	3
5	Susceptible	–		E1	–	–	E1	3	3
→ 4	Susceptible	–	3	3	3	3	3		3
1	Susceptible	E1	E1	E1	–	E1		3	3

* With reference to vaccine virus type fed
§ As in Table 8

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approximately 100,000 infectious units, all of the 3 strains were able to initiate infection in all but one of the presumably susceptible individuals. However, pharyngeal infection following doses of this size was exceptional, in contrast with the usual appearance of virus in the throat following larger inocula The small dose of 4,000 units of Type 1 virus failed to infect four persons and an equal dose of Type 3 may have been on the borderline of infectivity. All of the foregoing suggests that the infectivity of the attenuated strains may be considerably less than that of "wild" strains since the usual inoculum of the latter must be even smaller This conclusion is consistent, moreover, with the greatly reduced dissemination of the attenuated strains as compared with that of natural polioviruses in the same and similar families

Following infection in the recipient, however the sequence of events, in terms of the duration of viral excretion and serologic response, greatly resembles that which follows natural infection When it did occur, the rise in antibody titer, between the seventh and tenth day following ingestion in the majority, does not suggest that the previous sensitization with "Salk vaccine" had produced an accelerated response, although comparable studies in unvaccinated persons were not made

Among family units where spread of vaccine virus was studied, there were many variables relating to virus type and dose, socio-economic status, and age of the index person Perhaps even more important, concurrent infections with other enteric viruses and variations of contact within individual families may markedly influence the likelihood of viral transmission Conclusions on the influence of one or another of the known factors seem justified at this time therefore, only if the results are quite definite and consistent

There is a suggestion that Type 3 may spread more readily than either of the others, but, in some households Type 1 or 2 had 100% spread Virus dose was related only to the likelihood of resulting in pharyngeal excretion, which, in turn, was associated with somewhat more extensive transmission of Types 2 and 3 The significance of this observation is questionable since some contact infections occurred well after pharyngeal *excretion* had ceased. Also, a possible effect of the larger dose in suppressing or aborting coincidental "wild" virus infections or in resulting in a higher titer of virus being excreted in the feces, has not yet been evaluated. Therefore, the demonstration that spread occurs in the *absence* of pharyngeal excretion is of much greater import. This relates to the suggestion by Salk[5] that *if* formalinized vaccine reduces pharyngeal multiplication and *if* the transmission of poliovirus is by way of these excretions in a well sanitated population, then the widespread use of a formalinized vaccine may reduce the volume of transmission in such a community Spread did certainly occur by the fecal-oral route in many of these "Salk-vaccinated" families, and it did not occur among several upper economic families where the index person was a mother who was excreting virus from the throat

The one variable which definitely played a role in transmission was socio-economic status Only 8% of susceptible upper economic contacts became infected as contrasted with 51% among lower economic households This is a much greater difference than was seen in episodes of natural poliovirus transmission where the infection of almost all susceptible contacts is the rule, regardless of sanitary status Transmission outside the family unit to play contacts suggests the possibility that an entire community might be saturated with these strains if they were administered to a sufficiently large proportion of the children present. Despite their lower infectivity, the multiple opportunities for contact should enable them to compete effectively with other, "wild" polioviruses eventually, perhaps, to displace them

Interference between enteroviruses is a demonstrated laboratory phenomenon and has been postulated as an explanation for some peculiarities in the occurrence of epidemics of poliomyelitis and pleurodynia[6] Inhibition of vaccine virus spread by enteroviral interference is strongly suggested in certain of our unit studies If this occurred commonly, extensive community spread of vaccine polioviruses might be, paradoxically, limited both in poor environments, by viral interference, and in good environments, by superior hygienic standards

During this summer we will begin a study of possible community dissemination of vaccine viruses, when we feed only certain of the families, in two small communities in southern Louisiana

lected In addition and, as a measure of environmental contamination, flies were trapped at various points in the study area and elsewhere in the village. Flies were numerous in and around the privies, and were seen to feed frequently on feces exposed on the ground

Methods The specimens from this village which were eventually tested included *blood samples* obtained before the start of the trial and six weeks later; rectal swabs at 3-7 day intervals for 6 weeks, *fecal samples* obtained almost weekly from the outdoor privies belonging to the study group families and collections of *flies* also obtained almost weekly. Small as the study has been it has called so far for the examination of 163 blood specimens, 877 rectal swabs 302 privy samples and 238 collections of flies

Rhesus monkey kidney (MK) monolayer tissue cultures were used for the detection of virus Suckling mice were not used, and a number of Coxsackie A viruses may well have been missed

Agents isolated were identified by means of tube neutralization tests using hyperimmune sera against the various enteric viruses Mixtures of viruses were separated by several means including the plaque technique of Hsiung and Melnick [3] The immune status of the population to polioviruses was determined by means of neutralization tests on "pre" and "post" vaccinal sera, using the colorimetric method with disposable plastic panels [4] and in some instances the tube neutralization test based on inhibition of cytopathic effect

Some of the details of collecting and handling



Fig 2 Aerial view of part of the "isolated" village The study area is outlined with the heavy black line.

3. THE USE OF SABIN'S ATTENUATED TYPE 1 POLIOVIRUS VACCINE IN DIFFERENT ENVIRONMENTS, AND NEWER TECHNIQUES FOR TESTING THE VIRULENCE OF RECOVERED STRAINS *

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DR PAUL (*presenting the paper*) In several previous trials initiated by our laboratory,[1,2] we have observed and studied the ability of Sabin's attenuated Type 1 (LSc) poliovirus strain † to produce antibodies when used as an oral vaccine, and to spread to immediate contacts of the potential vaccines In a sense these have been observations in experimental epidemiology" in juvenile populations previously immunized with the Salk-type of formalinized vaccine In the first series of previous trials the population involved consisted of inmates of a cottage within an institution [1] Here the subjects were largely juvenile, moderately susceptible and apparently free from other enterovirus infections at the time when the live virus vaccine trials were being carried out In this so-called closed and "clean" setting the attenuated LSc strain spread rapidly and extensively from the vaccinees to those intimate contacts whose anti bodies had been acquired from Salk-type vaccine, but to few of those persons who had previously experienced natural infections

In the second trial,[2] the population was of a very different character, apparently highly resistant and heavily contaminated with other enteroviruses The setting was that of an open community representing a small semi isolated Indian settlement situated in southern Arizona The standards of living and sanitary conditions were of a low order and enterovirus infections were prevalent (Fig 1) Here one might have imagined that opportunities for spread to contacts of children fed the attenuated poliovirus were good, but this proved not to be the case Not only did the vaccine virus not spread to the immediate contacts of the few vaccinees, but only infected half of those to whom it had been orally administered



FIG 1 View of environmental setting in which the Arizona trial was carried out

The present report will be concerned with several observations made during this *second* trial (February-May 1958) and our attempts to interpret them Admittedly, the trial was very small It consisted of first outlining a "study area" within the village and selecting 21 Yaqui Indian families therein (Fig 2) On 13 February 1958, in 6 of these families a single child was fed Type 1 (LSc) poliovirus in a dose of 0 1 ml of tissue culture fluid containing 100,000 TCD_{50} In order to follow the subsequent course of events, as many rectal swabs and blood specimens were collected from the 131 members of the study group families as possible, and fecal samples from the family privies were also col

by

TABLE 1 COMPARISON OF MONKEY NEUROVIRULENCE AND THERMAL SENSITIVITY OF TWO TYPE I POLIOVIRUS STRAINS: MAHONEY AND LSc

STRAIN OF POLIOVIRUS	MONKEY INOC I-C (10^6-10^7TCD$_{50}$)		THERMAL REACT 39°C†			
			LOG-E O P	CLASS (b)		
	PARAL. (a)	LESIONS (a)		T	I	T+
MAHONEY (virulent)	(10/10)*	(10/10)*	−0 03			T+
			−0 2			T+
			−0 05			T+
			−0 4			T+
LSc (attenuated)	0/9	0/9	−5 96	T		
			−5 4	T		
			−6 2	T		
			−5 8	T		

(a) Monkey I C virulence
Denominator—Number tested
Numerator—Number positive

(b) Thermal marker
T—"avirulent"
I—intermediate
T+—virulent

* Results of test previously carried out in our laboratory
† Results of four separate tests

and possibly in one collection of flies, obtained at about the same time Coincidentally, and in keeping with the findings of others, it was apparent that our induced "epidemic" was in competition with other enteroviruses in the community In fact throughout the entire period of observation of more than two months many presumably wild polioviruses Type 1 and many obviously wild polioviruses Type 3, were encountered in the flies Similarly many non poliomyelitis enteroviruses were found in the rectal swabs obtained from the study population (Fig 4) and other members of the Village population, and also from the privies and from collections of flies, both of which latter sources were teeming with such viruses The viruses isolated included, in addition to poliovirus 1 and 3 and untypable agents, ECHO 1, ECHO 5, ECHO 10, and Coxsackie B5

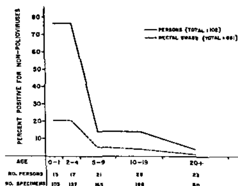


FIG 4 The percent of persons in whom rectal swabs proved positive for non polio myelitis enteroviruses as measured in age groups under 20 years of age

Virus isolations from flies Fig 5 shows the percent of enteroviruses per ml of flies collected at various stations in the village during the period from 19 February to 1 May 1958 It will be noted here that during the first two weeks of March the weather was unsatisfactory and the catches were light but after that they soon picked up The number of flies trapped, as measured in milliliters, the number of pools tested, and the number of positive pools are shown in this figure.

flies may deserve special mention. Shortly after the LSc strain was fed to the 6 children within the study area on 13 February 1958, twelve fly collecting stations were selected, 7 within the study area and 5 elsewhere in the village. Trapping of flies was started on 19 February, collections were continued about twice weekly until 1 May 1958. The traps containing flies were first placed in a large air-tight tin containing ether. The flies were then removed from the cage, put into sterile lusteroid tubes and immediately frozen in a box containing dry ice. Upon arrival at the laboratory in Phoenix, they were stored at −40°C until shipped to our laboratory in New Haven.*

No immediate attempt was made to separate the flies into individual species, as previous work published by this laboratory had demonstrated more than 15 years ago that poliovirus could be isolated from at least three separate genera of flies by monkey inoculation.[5] In general these are flies which feed on fecal material. In our present collections, in nearly all instances, the green bottle fly, *Phaenicia sericata*, made up at least 50 percent of the catch. The next most common genera noted were *Musca domestica*, *Muscina*, and *Phormia regina*, respectively. A smaller number of *Calliphora*, *Sarcophaga*, *Fannia*, and *Ophyra* were also present.† The techniques of preparing the flies for virus isolation and the identification of virus strains were similar to those used routinely in this laboratory for work of this kind. Rhesus monkey kidney (MK) tissue culture monolayers were employed.

Virulence tests were carried out on the recovered Type 1 poliovirus strains from the children who had been fed virus and became infected—i.e., from rectal swabs, from the privy of one family in which there was an infected child, and from flies trapped in the village. For the most part, second MK passage of these various isolates were tested. The methods used were: i) intracerebral inoculation of two monkeys with 1 to 10 x $10^{n}TCD_{50}$ of virus; and ii) thermosensitivity determinations as measured by the efficiency of plating (EOP) in agar overlay preparations incubated at various temperatures (35°, 37°, 39°C.), i.e., the use of a "thermal marker", which is a relatively new method of assessing virulence.[6,9] In the present studies, the pattern of behavior according to these two methods of detecting virulence of the strains isolated was compared with the behavior of the original, attenuated LSc strain which had been orally administered and the virulent Mahoney strain, the object being to determine whether the newly isolated strains were wild strains, or whether they represented strains with properties similar to the LSc strain which we had introduced into the community. See Table 1.

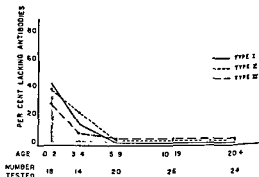


Fig. 3. The percent of children in the study area who lacked antibodies to Type 1 poliovirus appears as the shaded area. Estimates of those lacking other types of poliovirus antibodies can be made from the graphs for Type 1 and 2 respectively.

RESULTS

More extensive details of the results of the Arizona study are being reported elsewhere.[2] Some of the pertinent findings were as follows: Only 3 of the 6 children who received virus became infected and there was no spread to any of the familial or extra familial contacts. This was not surprising in view of the fact that virtually all persons over 5 years of age possessed antibodies to all 3 types—Fig. 3. Although attenuated virus did not spread to any of the exposed children it may have "contaminated" the community to the extent that it was probably picked up in one privy sample early in the trial

Wallis of the Connecticut Agricultural Station, New Haven, Conn.

sults indicate that there were actually waves of infection in the population, and these waves were to some extent reflected in the agents isolated from the privy samples and, although not illustrated here, also reflected irregularly in the fly specimens.

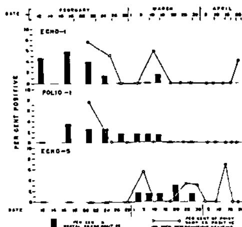


FIG 7 Comparative frequencies with which rectal swabs and privy samples were found to be positive for 3 viruses ECHO 1, Poliovirus 1 and ECHO 5, during the period February 12 to April 20, 1958

Virulence tests To identify the various Type 1 polioviruses encountered during the trial, tests for virulence were carried out (see Table 2) Most of the fly strains proved to be of relatively low virulence Thus 13 strains of Type 1 poliovirus isolated from flies and tested intracerebrally in 25 Rhesus monkeys in large doses produced typical but *mild* lesions in the central nervous systems of 23 of these animals, and 18 of the monkeys showed slight paralysis or transient muscle weakness One monkey died of pneumonia 6 days after inoculation but a Type 1 poliovirus was isolated in tissue culture from a suspension of its spinal cord Only one strain isolated from flies produced neither clinical nor pathological signs of poliomyelitis in two out of two animals inoculated

The correlation of monkey virulence with the thermal marker i e capacity of poliovirus to grow in tissue culture at 39°C., was remarkably good Thus the virus strains excreted by the infected children produced no weakness in any of the 10 monkeys inoculated and mild lesions in 1 of the 10 Correspondingly, the EOP at 39°C. of each of these strains was low and each could be classified as having the *T* marker The EOP of these strains at low bicarbonate concentration (0 08%), was also determined but the results were not so consistent, and will not be reviewed here

The 13 Type 1 poliovirus isolates from flies which produced lesions in monkeys, all showed a higher EOP at 39°C than did the LSc attenuated Type 1 virus used as a control, but they had a lower EOP than the virulent Mahoney type (Table 2). Thus of these 13 strains positive in monkeys, 5 were classified as *T+*, 7 as intermediate, and 1 as *T*. The Type 1 poliovirus isolated from flies on 20 February (10 01-20/II) had a low EOP at 39°C (*T*) and also failed to produce either symptoms or lesions when tested intercerebrally in rhesus monkeys Therefore it was similar to the LSc which has been orally administered but one cannot say that it was not a wild strain

COMMENT

The results of the Guadalupe Village Trial were meager in terms of induced poliovirus infection and spread of the vaccine virus to susceptibles. Statistically they prove very little On the other hand the observations emphasize the fact that immunity—or susceptibility—to poliovirus infection is a relative thing, and in crowded communities with primitive sanitation, where the level of resistance is high, both immunity and probably interference with other enteroviruses may prove to be an effective barrier to infection by attenuated virus.

Several ancillary findings of interest emerged in the trial. In spite of the fact that the trial was carried out in the winter months, there was no dearth of enterovirus infections in children and no dearth of these viruses in privies and flies In some instances the same agents were isolated from all three of these sources at roughly the same times, and the results provide a vivid picture of the extent of environmental contamination, infection, and immunization of susceptibles which come in waves within a community of this sort

The prevalence of enterovirus infections in the youngest children raises the question already raised by others as to whether interference might occur between various enteroviral agents

A surprising number of catches of flies were positive for polioviruses both Type 1 and Type 3. Most of the 25 Type 1 strains, and of the 32 Type 3 strains, the 6 which were tested, were of relatively low virulence. From a chronological standpoint, Type 1 poliovirus was most prevalent in flies during the early part of the trial (February and March), absent in April and again detected in one batch of flies collected on 1 May 1958 (see Fig. 5). Type 3 poliovirus was detected in a few lots of flies in February and March but more frequently in April. This

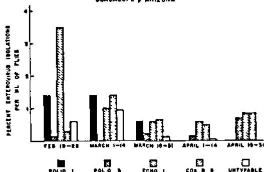


Fig. 5. Chronological distribution of various viruses detected in batches of flies trapped during the trial. Results expressed in terms of percent viruses present per ml. of flies.

prevalence and wide distribution of wild polioviruses in the community has seemed remarkable to us in view of the fact that there was so little evidence of human infection by these agents. It might almost seem as if the polioviruses were coming from an unknown source. Indeed, Type 3 poliovirus was isolated on only one occasion from this population, i.e., from the rectal swab of a child who was not in the study area. The specimen was *obtained from this child in March*, some 6 weeks after the trial had begun. No Type 3 poliovirus strains were isolated from the children at the time of peak isolation of Type 3 from flies.

Figure 6 shows the percent of isolations of various enteroviruses from flies trapped within the study areas as compared to isolations from flies trapped elsewhere in the village. It is apparent from these figures that there was no concentration of Type 1 poliovirus or other en-

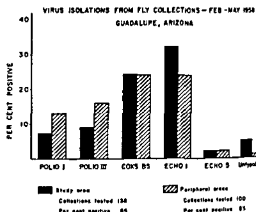


Fig. 6. Relative distribution of various enteroviruses found in flies trapped in the study area and elsewhere in the Village.

teroviruses in the study area as compared to the rest of the village.

The presence of so many enteroviruses in the community and in man has raised the familiar question of "heterologous interference" as a mechanism which might block infection with attenuated poliovirus and thus theoretically curtail the efficiency of an oral vaccine. If this occurs, it is important to know which enteroviruses are capable of acting as interfering agents. *Our* evidence that such a mechanism operated in this particular trial is only circumstantial. It is based primarily on the fact that the 3 children to whom the LSc virus was orally administered and who did become infected, were all members of families which were not coincidently infected with any other enteroviruses, whereas the three other children who failed to become infected when given the virus, were all members of families which were coincidently infected with ECHO 1 virus. In other words, the missed poliovirus infections occurred when the live virus vaccine was given in the presence *of a family epidemic of ECHO 1 virus infections.*

As an illustration of the multiple kinds of agents which were encountered in Guadalupe Village during the period of February-May 1958, the various enteroviruses which were detected from two sources (children and privies) have been charted in Fig. 7. ECHO 1 virus seemed to have been most prominent in February and early March. The same was true of the attenuated *LSc poliovirus 1*. *ECHO* 5 was isolated later during the period of observation. The re-

sults indicate that there were actually waves of infection in the population, and these waves were to some extent reflected in the agents isolated from the privy samples and, although not illustrated here, also reflected irregularly in the fly specimens

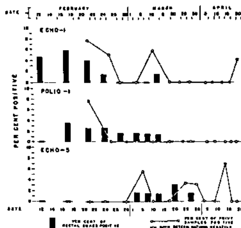


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TABLE 2 RESULTS OF TESTS FOR MONKEY NEUROVIRULENCE AND THERMAL MARKER ON 19 TYPE I POLIOVIRUS STRAINS ISOLATED DURING A VILLAGE FIELD TRIAL

Source of Poliovirus	Site	Date	Monkey Inoc I-C (10^6–$10^7 TCD_{50}$)		Thermal React 39°C			
			Paral	Lesions	Log —E O P.	Class		
	Family					T	I	T+
Excreted by Infected Children	23–8	Feb 17	0/2	0/2	−5.5	T		
	23–8	Feb 21	0/2	0/2	−5.3	T		
	20–8	Feb 18	0/2	0/2	−6.0	T		
	20–8	Feb 20	0/2	0/2	−5.0	T		
	10–5	Feb 27	0/2	1/2	−4.1	T		
	Family							
Privy of Fam A	20–A	Feb 20	0/2	1/2	−4.0		I	
	Trap							
	10–01*	Feb 20	0/2	0/2	−4.8	T		
Flies Trapped In Village	6–01	Feb 27	0/2	2/2	−3.5		I	
	1–26	Mar 20	2/2	2/2	−3.4		I	
	1–26	Feb 19	1/2	2/2	−3.2		I	
	10–01*	Feb 19	1/2	2/2	−2.8		I	
	13–03	Feb 25	2/2	2/2	−2.4		I	
	1–26	Feb 20	1/2	2/2	−2.4		I	
	1–26	Feb 27	2/2	2/2	−2.2		I	
	7–13*	Feb 27	2/2	2/2	−2.0			T+
	6–01	Mar 6	2/2	2/2	−1.8			T+
	6–01	Mar 11	2/2	2/2	−0.9			T+
	3–06	Mar 20	1/1	1/1	−0.7			T+
	3–06	Mar 11	2/2	2/2	+0.2			T+

* Trap site within study area Other legends as in Table 1

and polioviruses to a degree sufficient to hamper the efficiency of oral vaccination with attenuated polioviruses In other populations living in similar or better environments than that existing in the village in which our trial was conducted, similar high enteric infection rates have been found among the children 0-4 years of age Since these are the members of such populations who are candidates with a high priority for vaccination against poliomyelitis, the question of interference between competing agents is one which cannot be ignored The results of the present study suggest that there is competition, and perhaps recent ECHO 1 virus infection may have had a part in preventing infection with the poliovirus fed; that this can occur is supported by recent observations by Gelfand, Fox *et al* [10]

It was impressive that flies were already teeming with viruses in February and March, and many more polioviruses were isolated from them than from children This observation again raises the question as to how important flies might be conceivably in reducing the susceptibility of populations to poliovirus and other en

teroviruses, by repeatedly making available in one way or another small immunizing doses of these agents In any event it would seem that there is still much to be learned here. Our small village field trial limited to one strain, emphasizes the complexity of the problems of community susceptibility, the competition with wild strains of virus and the many environmental factors which may be important in interpreting the over all picture

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DISCUSSION

CHAIRMAN STUART-HARRIS: The papers presented by Dr Gelfand and Dr Paul are open for discussion.

DR ABAD GOMEZ I would like to ask if Dr Langmuir knows how much virus was excreted from the children vaccinated, how many contacts received that virus, and of those contacts who received the virus, how many did develop paralytic polio

DR LANGMUIR I do not have the data in great deal, but I think we can say we do have the *basic number of doses of Cutter vaccine given* A little over 300,000 doses were given in the school clinics, to first and second grade school children While there might be a little overlap there in families with children close together, this represents close to 300,000 typical average American families that participated in this program

In addition, somewhere from 40 to 80 thousand doses of vaccine were distributed through commercial channels to private physicians largely and here a good many multiple inoculations in the same family were probably given

We can make certain calculations in that 104 contact cases occurred and these occurred in families where children were given the vaccine Only one of these children who got the vaccine *developed polio, that was a non-paralytic case* There were 79 vaccinated cases, and only 1 overlap. To make a very rough or crude calculation, if you multiply those two together, you come up with a crude estimate of approximately 7,000 infectious foci created by the vaccine

We have no idea what the dosage was in any sense comparable to tissue cultures

CHAIRMAN *STUART HARRIS: Does that answer* your question, Doctor?

DR ABAD GÓMEZ: The answer is, Dr Langmuir, that of 7,000 foci created, you got 105 paralytic cases.

DR. LANGMUIR I think 80 were paralytic and 25 non-paralytic

DR SABIN: Am I correct in assuming that the amount of virus excreted by the intramuscularly vaccinated children is not known; and is it possible that the amount of virus excreted after the *secondary localization from the blood stream* may not be the same after the initial infection of the intestinal tract? If there is any information on this, I think it might help to understand the issue raised by Dr. Abad Gómez

DR. LANGMUIR. I do not know whether titers were done We are well aware that there were a good many localized outbreaks following vaccinated individuals—Dr. Shelokov studied one outside of Baltimore There was a study in a Colorado nursery school As I remember, most of the children were infected following intradermal inoculations of the vaccine How much of this was direct from the vaccine or from one infected individual spreading secondarily, we cannot say We certainly have no estimates of *titers comparable to what we are now dealing* with in our present live virus vaccines

Others in the room I am sure, may have something to offer in this respect

DR. ANDERSON I was simply worried about some of Dr Langmuir's calculations, when he talked about the number of persons exposed Is he forgetting the fact that the Cutter incident was with only two of the lots of Cutter vaccine, and that there were about 400,000 cc or doses that got out, about 300,000 for clinic use and about 100,000 for use in private practice?

Personally I am not aware of the actual size of those particular lots that were infected Perhaps Dr Langmuir knows the size of those. The calculations, I think, should be based upon those two lots and not upon the total

DR LANGMUIR I agree with Dr Anderson completely. I mentioned this morning that my best estimate, from my memory only, would be that the two lots were large and would amount to about 100,000 doses of vaccine. This is subject to confirmation. I agree heartily that we should think in terms of those, and the 7,000

fact that I estimate in this other very quick calculation would come out of this group.

Let me also comment that the contact cases came from families exposed and that while that is 100,000 doses, there would be, whatever the average family size in the country is, a factor to multiply here for the number exposed to the opportunity of Cutter infection

DR. ANDERSON There is another factor, I think, that should be remembered, if we are trying to estimate probable infections The cases that occurred on a clinical basis in Idaho were out in the rural areas Some of them were so rural that you could not even find them on a road map Within the larger cities of Idaho such as Moscow and Boise, the same lots were used without any cases developing, thus indicating it was not simply a question of was there live virus in the vaccine, but also a question in terms of the resistance of the individuals

Most of the cases occurred in the highly isolated areas, where there would have been little poliomyelitis in the past That is borne out when you see the very low rates of Idaho for the two or three years preceding the Cutter incident

DR. FOX: I wanted to go back to the question that Dr Plotkin asked Dr Bodian originally What did he think would happen if chimpanzees were fed Mahoney virus in the same dosage as the tissue culture strains? I think that really was the crucial question I do not think one can assume that the dosages that were passed around by natural means, once the virus had been unnaturally introduced into the population through the vaccine, are necessarily to be compared with the tissue culture doses which are fed in the oral vaccine trial

DR BODIAN I would just like to reply that we have not fed enough chimpanzees with the Mahoney strain to be able to answer this question We have fed virulent Mahoney virus to susceptible chimpanzees in doses which we thought were adequate to produce a certain degree of paralytic disease We have failed to obtain a single paralytic case following feeding with Mahoney tissue culture virus, which was virulent in monkey tests

We also fed the Wallingford strain to chimpanzees, and in one group of nine, reported some time ago there were two paralyzed and two with non paralytic polio, but another group of ten susceptible chimpanzees were fed the same material with no resulting paralytic or non-paralytic cases.

We can deduce from this type of information that there are considerable variations to be seen in the susceptibility to paralytic polio upon feeding chimpanzees

We must remember that in households infected during the severest epidemics in this country the secondary attack rates run around 1 to 5 per cent We do not need the Cutter incident to get some sort of measure of paralytic capacity of virulent strains. We have to conclude that by the oral route in the general population, with a few astonishing exceptions, such as the Hudson Bay outbreak, we have a low risk in terms of the numbers of chimpanzees that we have discussed today or the number of individuals that Dr Koprowski presented Thus even with the most virulent strains that we know anything about, it is clear that by feeding, one cannot expect to obtain high paralytic rates

DR COURTOIS In trying to infect chimpanzees orally and in trying to give about 10 cc of a tissue culture fluid containing 10^7 $TCID_{50}$, we get 5 paralyzed out of 25, and with YSK strain of polio (Type 2), and with the strain "Mexican" (Type 1) 4 out of 25 All this with an oral infection

DR SABIN: I would like to add to this I believe Dr Bodian forgot to mention his own experience together with Dr. Holland, that with the Brunhilde virus particularly before tissue cultures using the infected monkey spinal cord which might give quite different results, I admit the situation was comparable to that just mentioned by Dr Courtois namely, 20 per cent

I would like to say that in the experiments I reported some years ago, the feeding of Mahoney virus after small number of passages in tissue culture, regularly produced paralysis in 60 to 70 per cent of cynomolgus monkeys infected by the oral route excluding those who had been infected by accidental contamination of the olfactory bulb

This was a figure, I think, reported this morning by Dr Plotkin on the more recent studies of Craig and Brown

DR. PAUL: There are two people in this room who have considered this problem from another angle. Dr Melnick, in his studies on clinical case rates in relation to infection rates; and, if Dr. Gard has Dr Olin's figures from Sweden available, they also bear on the problem

DR MELNICK: With regard to Dr. Paul's comments, our data on this point were obtained by studying the acquisition of poliomyelitis antibodies during a severe Type 1 epidemic in North Carolina in 1948. This allowed us to determine subclinical infection with a virulent epidemic strain The analysis (*American Journal of Hygiene, 58:* 207, 1953) showed that one paralytic case occurred in approximately 100 children infected with the epidemic virus.

Another approach to this has been through the analysis of infections with the virulent Mahoney virus in the Cutter material Two studies, one by the neutralization test and one by the complement-fixation test, indicated that high antibody titers were present in 31 to 40 per cent of children 6 to 8 weeks after receiving a single intramuscular dose of the vaccine containing live virus, in contrast to 1 to 8 per cent in control, non-vaccinated children in the same area, or in children receiving a single dose of properly inactivated Salk vaccine As 20 Type 1 (virulent Mahoney strain) cases occurred among 32,000 children vaccinated with the inadequately inactivated lots, the serological data indicate that 20 cases occurred among some 10,000 infected children, or 1 case per 500 infected

DR GARD: On attack rates in the susceptible individuals, based upon comparison between actual recorded attack rates and immunological rates, as revealed from immunological surveys of the population, I would like first to call attention to recent statistics from Israel, where quite severe epidemics in the under five-year age group had occurred for a number of years. The immunological conversion rate indicates an average rate of infection in this population of the order of 20 to 25 per cent, per year.

Now, the paralytic attack rate in the age group under one year was last year 460 per 100,000, that is .46 per cent If that is multiplied by a factor of 4 or 5, to correct for actual infection rates, we arrive at the figure of about 2 out of 100 infected individuals who would develop paralysis.

In Sweden Dr. Olin, covering a period of 25 years, and comparing accumulated attack rates with immunological conversion rates, estimated the risk of developing paralysis after the first exposure to Type 1 virus to about 3 per 1,000 in the age groups below 5 years But the risk increases rapidly with age So that a susceptible individual in age groups of 25 or above runs from 20 to 100 times greater risk to develop paralysis than does the infant.

It should be remembered that Olin's data were collected over a period of 25 years They represent an average of all the strains that have appeared in the country during that time. Presumably these estimates come fairly close to what can be expected from moderately virulent strains.

TOPIC IV. THE PROBLEM OF INTERFERENCE IN LIVE POLIOVIRUS IMMUNIZATION

1. IMMUNOLOGIC RESPONSE TO TRIVALENT ORAL POLIOMYELITIS VACCINE

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DR COX *(presenting the paper)* A survey of the literature to date on feeding living attenuated polioviruses for the immunization of man reveals that relatively little has been reported on feeding the three types of virus simultaneously or using *mixtures of such as a trivalent vaccine* This is apparently due to the fact that certain strains of poliovirus were found to interfere with the establishment and multiplication of other type strains in the human gut Thus, Koprowski [1] reported that Type 1, SM virus, interfered with the immunizing effect of Type 2, TN strain when the two were fed simultaneously In a subsequent study Koprowski and his associates [2] attempted to overcome this interference by feeding much larger doses of Type 2 virus in a mixture with Type 1 Again interference was found to occur between the two types, although under the conditions of the latter study Type 1 strain was not always dominant In 1955 Sabin [3] stated that simultaneous feeding of approximately 10 million tissue culture 50%-infective doses (TCD_{50}) of attenuated polioviruses of all three types to chimpanzees completely suppressed the multiplication and immunizing effect of only Type 3 virus In 1956 Sabin reported [4] that when he fed approximately one million TCD_{50} of the naturally occurring attenuated strains (P 2149, P 712, and Glenn) simultaneously, he found no significant interference in four volunteers fed a mixture of Types 1 and 2, nor in three volunteers fed a mixture of Types 1 and 3, nor in three persons fed Types 2 and 3 nor in one person fed all three types together A delay in appearance of antibodies as well as *titers in the lower range were noted* in some of the individuals fed In later studies carried out with his "optimum single plaque" strains, Sabin reported [5] that the Type 2 strain was the dominant one and that quite often, although not always, it interfered with the multiplication of the other two types when fed as a mixture This finding prompted him to state that "immunization against all three types of poliovirus by a single administration of a mixture of them is not feasible" [6] Sabin therefore, recommended that his three "optimal" strains be fed separately at 3 week intervals in the order of Types 1, 3, and 2

In contrast to some of the above findings Smorodintsev and his associates working with some of Sabin's earlier strain variants (Type 1 LS of 12-22 55, Type 2 P 712 10ab of 9-5-56, and Type 3, Leon 14ab of 4-5 56) have recently reported [7] a single experiment in which a trivalent vaccine containing one million TCD_{50} (a mixture of equal volumes of monovalent strains) was fed to eight staff members of the Institute's Virology Department * and to 29 children, 7 to

* Institute of Experimental Medicine, USSR Academy of Medical Sciences, Leningrad

15 years old. No comments are made about the serological results obtained in the eight staff members but the children with low or medium antibody levels before immunization showed "an increase in antibodies to all three types of virus"

Our interest in the possibility of using a trivalent vaccine for simultaneous immunization of humans against all three types of poliomyelitis by a single feeding was renewed about a year ago when one of us (M R-C.) found that it was possible to maintain a mixed infection of Types 2 and 3 polioviruses for at least 20 serial passages in monkey kidney tissue culture. Both types of virus were present in the stool specimen of a Minnesota child who had been fed the Lederle strains of attenuated polioviruses[8] This observation suggested that it might be possible to immunize against the three types of poliomyelitis by feeding the Lederle strains either in the form of capsules taken simultaneously or as a mixture in a stabilized fluid preparation, and a number of studies were initiated Other studies in this connection will be reported independently

This paper presents results obtained to date in feeding different dosages of a liquid, trivalent, oral, poliomyelitis vaccine to approximately 550 persons, principally employees of Lederle Laboraties, Pearl River, New York, who voluntarily requested to be vaccinated, and in a few cases, members of their immediate families All these individuals live in a semi-urban area located within approximately a 15-mile radius of Pearl River, New York, chifly Bergen County (northern New Jersey) and Rockland County (southern New York). The study was well controlled since all persons involved had ready access to the Medical Department of Lederle Laboratories, so that a good history and follow-up was obtainable in each case The data presented here consist of the results of a serologic study carried out on 241 persons out of the 550 fed, however, we now have informative data on 286 additional vaccinated persons, and if Dr Stuart Harris believes that there is sufficient time, I can read a summary of these data at the end of the paper

CLINICAL OBSERVATIONS

Even though a very careful follow-up was made, only 3 individuals of the 550 persons who were fed reported any clinical signs of illness *during* the observation period of more than 2 months At the time of the second bleeding, one individual reported that on the 7th or 8th day following ingestion of the vaccine, she had a rather severe headache, associated with stiffness of the neck, lasting for approximately 24 hours During this time the patient felt flushed but remained afebrile and had no sore throat This condition cleared up spontaneously, rather abruptly, and no further sequelae have been reported Serologic testing revealed this person to have been triple negative before vaccination and to have responded to Types 1 and 3 polio virues.

The second and third patients experienced diarrhea which lasted for a day or two during the third and fourth week, respectively, following ingestion of the oral vaccine. Since a diarrheal condition was being reported frequently in unvaccinated subjects in the community, it is believed that these latter cases should be completely dismissed as being associated with the vaccine

LABORATORY DATA

Materials and Methods

Strains of Virus The viruses used in this study were the Lederle strains SM (Type 1), MEF_1 (Type 2), and Fox (Type 3) isolate No P 1149 These are the same strains which were used previously in studies carried out in Minnesota, Colombia, Uruguay, and Cuba. The properties and behavior of these strains are now being reported separately by Cabasso and his associates[9]

Neutralization Test Neutralization tests were carried out on the pre- and post-vaccination sera simultaneously, using the pH or color method according to the procedure of Salk and Youngner[10] All sera were first inactivated for 30 minutes at 56°C in a constant temperature water bath The serum samples were then prepared in four-fold dilutions in duplicate, using 0 25 ml per dilution Approximately 100 to 300 TCD_{50} of the representative strains of virus per 0 25 ml were added to the respective serum dilutions and the mixtures were held at room temperature for 3 hours Trypsinized monkey-kidney-tissue cell suspensions containing approximately 600,000 cells per 0.25 ml. were then added to each of the serum-virus mixtures along with appropriate controls and the tubes then held at 37°C until the sixth or seventh day when they were read. Antibody titers were calculated by the method of Reed and Muench[11] Antibody titers

TABLE 1 ANTIBODY RESPONSE OF 10 PERSONS FED 0 5 CC OF TRIVALENT ORAL POLIOMYELITIS VACCINE ($10^{7.5}$ TCD_{50} EACH TYPE VIRUS PER DOSE)

SUBJECT	AGE (YEARS)	VACCINE FED	BLEEDING DATE	ANTIBODY TITERS		
				TYPE 1	TYPE 2	TYPE 3
#1	24	*	6/12/58	<1 4	<1 4	<1 4
			9/13/58	1.512	1 256	1 64
#2	36	*	6/12/58	<1 4	1.512	<1 4
			9/12/58	1 256	>1 1024	1 32
			3/ 4/59	1 512	>1 1024	>1 1024
#3	12	7-1238-801†	11/28/58	<1 4	<1 4	1 32
			2/ 3/59	1 128	1 8	1 512
#4	18	"	11/28/58	1 32	1 128	1 8
			2/ 3/59	1 512	1 512	1 128
#5	29	"	11/28/58	<1 4	<1 4	1 512
			1/29/59	1.32	1 128	>1 1024
#6	29	"	11/28/58	1 512	<1 4	<1 4
			1/21/59	>1 1024	<1 4	1 128
#7	29	"	11/28/58	<1 4	1 8	<1 4
			1/21/59	1 128	1.32	1 8
#8	5	"	1/13/59	<1 4	1 128	1 8
			2/15/59	1.32	>1024	>1 1024
#9	10		1/13/59	<1 4	1 512	1 8
			2/15/59	1 128	>1 1024	1 512
#10	13		1/13/59	1 512	1 512	1 32
			2/15/59	1 512	1 512	1 512

* A mixture of equal volumes of Type 1 (7 1231 121), Type 2 (7 1232 216), and Type 3 (7-1233 318)
† A mixture of equal volumes of Type 1 (7 1231 115), Type 2 (7 1232 217), and Type 3 (7 1233 319)

of less than 1 4 were considered negative, whereas antibody titers of 1 4 or greater were considered as positive Sero-positive persons showing a four-fold or greater increase in antibody titer as a result of vaccination were counted as having had a booster effect

RESULTS

Results of the first experiment using a liquid trivalent vaccine, carried out on two individuals in June 1958, are shown in Table 1 (Subject Numbers 1 and 2) *Subject No 1, 24 years* old, was a triple negative (no antibodies against any of the three types of poliovirus) Subject No 2 showed no antibodies against two types of poliovirus (1 and 2) Titration of the post-vaccination sera, carried out approximately 3 months later, indicated that both individuals responded well to the vaccine as shown by the antibody levels attained against all three types of poliovirus Further, it is seen from the results obtained on the blood sample taken in March 1959 on Subject No 2 that these antibodies persisted for at least 9 months Each of these individuals *was fed liquid vaccine consisting* of equal volumes of each of the three types of polio-

virus, and the dosage was approximately $10^{5.5}$ (300,000) TCD_{50} of each type of virus. Eight additional persons were fed a second preparation of liquid trivalent vaccine bearing the Lot No 7 1238-801 and containing approximately $10^{5.5}$ (300,000) TCD_{50} of each type virus per 0.5 ml. This vaccine has been used for all the additional trials reported in this paper From data presented in Tables 1 and 2, it is seen that a good antibody response was induced in each individual by the 0 5 ml. dose of vaccine These include one person who was a triple negative, two who were double negatives for Types 1 and 2, one who was a double negative for Types 2 and 3, two who were double negatives for Types 1 and 3, and two additional persons who were negative for Type 1

After feeding the trivalent vaccine, only 1 person was left without demonstrable antibodies to any one of the three types of poliovirus (see Table 2) Prior to vaccination this individual had been a double negative for Types 2 and 3 poliovirus He apparently failed to respond to the Type 2 component of the vaccine No persons were left as double or triple negatives after being fed the trivalent preparation

Table 3 shows the results obtained in a single individual, 38 years old, who first was fed capsules containing approximately $10^{5.0}$ (100,000) TCD_{50} of each of the three types of poliovirus at 3-week intervals This man who showed no antibodies against Types 2 and 3 poliovirus, failed to get an immunogenic response following this feeding procedure Subsequently, in November 1958, he was fed a 0 5 ml. dose of liquid trivalent vaccine, and again he failed to show a demonstrable serologic response. Finally, in January 1959, he was fed a 2 0 ml. dose of the same liquid vaccine which contained approximately $10^{6.1}$ (1,200,000) TCD_{50} of each of the three poliovirus strains. Titration of the serum sample taken on 11 February 1959, showed a good serologic response for all three types of poliovirus, antibody titers were 1 1024 for Type 1, 1 512 for Type 2, and 1:32 for Type 3.

Following these encouraging results, a comparative study was set up in which one group of patients received a 1 0 ml. dose of trivalent vaccine representing $10^{5.8}$ (600,000) TCD_{50} of each strain of poliovirus, while a second group received a 2 0 ml dose representing $10^{6.1}$ (1,200,000) TCD_{50} of each type. Table 4, Charts 1, 2, 3, and Fig 1 summarize the data which were obtained on the subjects who received the 1 0 ml dose of trivalent vaccine, Lot No 7-1238-801 These 42 volunteers ranged in age from 8 to 65 years with the majority between the ages of 11 and 50 Among this group there was one triple negative, 2 double negatives for Types 1 and 2, one double negative for Types 2 and 3, three double negatives for Types 1 and 3, a single

TABLE 2 PRE- AND POST-VACCINATION STATUS OF 8 ANTIBODY NEGATIVES AMONG 10 PERSONS FED 0 5 CC TRIVALENT ORAL POLIOMYELITIS VACCINE ($10^{5.5}$ TCD_{50} EACH TYPE VIRUS PER DOSE)

Status of Subjects	Negative for Types			Age in Years	Post-Vaccination Results Negative for Types
Triple negative 1	1	2	3	24	0
Double negative 5				12, 29	0
2	1	2		12, 29	0
1		2	3	29	2
2	1		3	29, 36	0
Single negative 2	1			5, 10	0
Total: 8	Sum total all negatives Pre-15, Post-1				

TABLE 3. ANTIBODY PATTERN OF AN ADULT FOLLOWING THE FEEDING OF DIFFERENT DOSAGES OF ORAL POLIOMYELITIS VACCINE (DR. A T., 38 YEARS)

Vaccine	Material Fed	Virus Titer of Vaccine	Pre- or Post-Feeding	Bleeding Date	Antibody Titer†		
					Type 1	Type 2	Type 3
Each of the 3 polio types at 3-wk intervals	Capsules	$10^{5.6}$ TCD_{50} per capsule	Pre	9/ 9/58	32	<4	<4
			Post	10/20/58	64	<4	<4
0 5 cc Trivalent #7-1238-801*	Liquid	$10^{5.5}$ TCD_{50} each virus type	Pre	11/19/58	128	<4	<4
			Post	12/19/58	128	<4	<4
2 0 cc Trivalent #7-1238-801	Liquid	$10^{6.1}$ TCD_{50} each virus type	Pre	1/ 5/59	128	<4	<4
			Post	2/11/59	1024	512	32

* #7-1238-801=equal volumes of Type 1 (#7 1231 115), Type 2 (#7-1232 217), and Type 3 (#7 1233-319)
† Reciprocal of the serum dilution

TABLE 4 PRE- AND POST-VACCINATION STATUS OF 16 ANTIBODY NEGATIVES AMONG 42 PERSONS FED 1 0 CC OF TRIVALENT ORAL POLIOMYELITIS VACCINE ($10^{5.5}$ TCD_{50} EACH TYPE VIRUS PER DOSE)

Pre-Vaccination Antibody Negatives		Post-Vaccination Status			
Negative to Type	No of Subjects	Negative to Type			Converted
		1	2	3	
1, 2, 3	1			1	
1, 2	2				2
1, 3	3	1			2
2, 3	1				1
1	1	1			
2	5		3		2
3	3			1	2
	Totals 16	2	3	2	9

Sum Total all negatives Pre-24, Post-7

negative for Type 1, five single negatives for Type 2, and three single negatives for Type 3. Thus there were 16 persons showing a total of 24 "negatives" for all three types of poliovirus. See Table 4 which also gives the post-vaccination antibody pattern of these 42 persons approximately 30 days after feeding the 1.0 ml dose of trivalent vaccine, note that no triple or double negatives remained after vaccination and only seven persons were left as single negatives for any of the three types of poliovirus. One of these subjects remained without antibody to Type 1, he previously had been a double negative for Types 1 and 3. A second individual, originally a single negative to Type 1, was left without antibodies to Type 1. Three persons, previously negative to Type 2 virus only, still were without antibodies to this type virus. One individual who had been a triple negative was left without antibodies to Type 3 virus only, and one individual who previously was a single negative for Type 3 virus did not respond to the Type 3 component of the triple vaccine. For detailed information concerning individuals who were fed the trivalent vaccine see Charts 1, 2 and 3, which depict the antibody response of these 42 persons to Types 1, 2 and 3 polioviruses, respectively.

Figure 1 shows the number and percentage of persons who demonstrated a four-fold or greater antibody rise following the feeding of the 1.0 ml dose of trivalent vaccine. While the numbers are small, it is seen from this figure that, of those individuals with pre-feeding antibody titers of <1:4, 71% responded to Type 1, 67% to Type 2 and 75% to Type 3. Of those with antibody titers of <1:16 either a primary or a booster response was shown by 70% to Type 1, 72% to Type 2 and 82% to Type 3. As would be expected a decreasing proportion of those persons with greater initial antibody titers manifested a response, but even of those persons showing initial antibody titers <1:1024, 35% showed either a primary or a booster effect to Type 1, 47% to Type 2 and 54% to Type 3.

The response among these volunteers should be compared with that obtained in 188 volunteers fed the 2.0 ml. dose of the same vaccine, which represents $10^{6.1}$ (1,200,000) TCD_{50} of each type virus per dose. See Table 5 and Charts 4, 5 and 6. The age range of the 188 persons was from 4 through 60 years, with the greatest numbers ranging from 21 through 50. In this group, there were 11 triple negatives (aged 18 to 41), 18 double negatives to Types 1 and 2 (17 to 39), 7 double negatives to Types 2 and 3 (19 to 38), and 10 double negatives to Types 1 and 3 (23 to 54). There were 14 single negatives to Type 1 poliovirus (20 to 50); 6 single negatives to Type 2 (22 to 39); and 30 single negatives to Type 3 (10 to 58). Thus there were 96 persons showing a total of 153 "negatives" for all three types of poliovirus. Results obtained when the sera of these same individuals were tested approximately 30 days after feeding the 2.0 ml dose of trivalent oral poliomyelitis vaccine are shown in Table 5. No triple or double negatives and only 11 single negatives remained. Two individuals were still negative to Type 1, nine were left negative to Type 2; and none remained negative to Type 3. The pre-vaccination status of those who failed to show an antibody response to the triple vaccine is presented in the lefthand column of Table 5. One of the persons left without antibody to Type 1 had been a single negative to Type 1. The second individual left without antibody to Type 1 had been a double negative to Types 1 and 3. One of the subjects who remained negative to Type 2 had been a single negative to Type 2. Another person left without Type 2 antibody had been a Type 2 and 3 double negative. In addition three persons who were originally Types 1 and 2 double negative and four persons who were triple negatives were left without Type 2 antibody. Charts 4, 5 and 6 show the antibody response of the 188 persons fed the 2.0 ml. dose of the trivalent vaccine to Types 1, 2 and 3 polioviruses.

A summary of antibody conversion effect as well as booster response of the 188 persons fed 2.0 ml of vaccine is shown in Figure 2. Of those individuals with antibody titers originally <1:4, 96% responded with antibodies to Type 1, 78% to Type 2 and 100% to Type 3. Likewise, of all individuals who showed pre-vaccination antibody titers of <1:16, 90% showed either a primary or booster effect to Type 1, 83% to Type 2, and 95% to Type 3. Corresponding primary or booster effects are shown for those groups of individuals whose initial antibody titers were <1:64, <1:256, or <1:1024. It is remarkable to note that of all persons who showed initial antibody titers <1:1024, 62% showed either a primary or a booster response to Type 1, 67% to Type 2, and 71% to Type 3.

CHART I Poliovirus Type 1 Antibody Response of 42 Persons Fed 1 0 cc of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

Pre-Feeding	Antibody Titers — Post-Feeding <1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024	Total Number Fed	No With 4-Fold or Greater Response
<1 4	2				4		1					7	5
1 4			1									1	0
1 8							1	1				2	2
1 16				1	1						1	3	1
1 32					1	1		1	1	1		5	3
1 64						1						1	0
1 128							2			1		3	1
1 256							1	5	2	1		9	1
1 512								1	4	1		6	0
1 1024									1			1	
>1 1024											4	4	
Totals	2	0	1	1	6	2	5	8	8	4	5	42	13

Chart 2. Poliovirus Type 2 Antibody Response of 42 Persons Fed 1.0 cc of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

Antibody Titers												Total Number Fed	No. with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	3	3	1		1				1			9	6
1:4									1			1	1
1:8									1			1	1
1:16				1	1							2	0
1:32				1	1					1		3	1
1:64											1	1	1
1:128							1	1	1		1	4	2
1:256							1	2	1			4	0
1:512								1	3	1	4	9	1
1:1024										1	1	2	
>1:1024									1		5	6	
Totals	3	3	1	2	3	0	2	4	9	3	12	42	16

Chart 3 Poliovirus Type 3 Antibody Response of 12 Persons Fed 1.0 c.c. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

Pre-Feeding	Antibody Titers — Post-Feeding											Total Number Fed	No. With 4-Fold or Greater Response
	<1:1	1:1	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:1	2	1	2		2				1			8	6
1:4				1								1	1
1:8							1	1				2	2
1:16					2			1			1	4	2
1:32							1		1	1		3	1
1:64								1			1	2	2
1:128							3	2	2		1	8	3
1:256							3	1				4	0
1:512									3	1	1	5	1
1:1024										2		2	
>1:1024										1	2	3	
Totals	2	1	2	1	1	0	8	6	7	5	6	42	20

Chart 2. Poliovirus Type 2 Antibody Response of 42 Persons Fed 1 0 cc of Trivalent Oral Poliomyelitis Vaccine ($10^{5\,8}$ TCD_{50} of Each Type per Dose)

Antibody Titers												Total Number Fed	No With 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024		
<1 1	3	3	1		1				1			9	6
1 4									1			1	1
1 8									1			1	1
1 16				1	1							2	0
1·32				1	1					1		3	1
1 64											1	1	1
1 128							1	1	1		1	4	2
1 256							1	2	1			4	0
1 512								1	3	1	4	9	4
1 1024										1	1	2	
>1 1024									1		5	6	
Totals	3	3	1	2	3	0	2	4	9	3	12	42	16

Certainly use of the trivalent vaccine as reported here gave excellent results indeed in immunizing against Types 1 and 3, and although the rate of response to Type 2 was not as good, it is nevertheless encouraging inasmuch as these results were obtained by feeding adults a single 2.0 ml dose

It must be pointed out also that in this study the vaccine was fed at various times of day and no special effort was made to determine if a better rate of response could be obtained by feeding the vaccine under more optimal conditions, such as before or after meals Perhaps feeding the vaccine after a meal, at which time the gastric acidity could be partially reduced by food, might give better results Perhaps an even higher rate of response could be obtained by giving a second dose of trivalent vaccine at a later interval such as 6 to 8 weeks after the administration of the first dose. Such a procedure might fill in the "negative gaps" among those individuals who failed to respond to the first feeding

These problems are being explored at the present time

Finally, similar studies are now underway with trivalent vaccines prepared from sub-line strains derived from the strains of poliovirus discussed here These particular sub-line strains seem to show less neurotropic activity upon inoculation into monkeys and their immunogenicity for man is under investigation In any event, there seems to be no doubt whatsoever, that by using the strains of poliovirus reported here, it is entirely possible to give rise to a high state of immunity in adults, and presumably in children also, to all three types of poliovirus *by a single feeding* of trivalent oral poliomyelitis vaccine

SUMMARY AND CONCLUSIONS

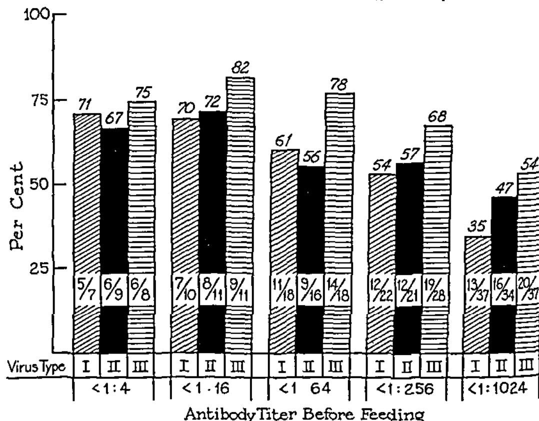
To date a serologic study has been completed on 211 paired sera obtained from 550 volunteers fed various dosages of a liquid trivalent poliovirus vaccine The majority of subjects in the study were employees of Lederle Laboratories

TABLE 5 PRE- AND POST-VACCINATION STATUS OF 96 ANTIBODY NEGATIVES AMONG 188 PERSONS FED 2.0 CC OF TRIVALENT ORAL POLIOMYELITIS VACCINE ($10^{8.1}$ TCD_{50} EACH TYPE VIRUS PER DOSE)

Pre-Vaccination Antibody Negatives		Post-Vaccination Status			
		Negative to Type			
Negative to Type	No of Subjects	1	2	3	Converted
1, 2, 3	11		4		7
1, 2	18		3		15
1, 3	10	1			9
2, 3	7		1		6
1	14	1			13
2	6		1		5
3	30				30
	Totals 96	2	9	0	85

Sum total all negatives Pre-153 Post-11

Fig 1 Percentage of persons with fourfold or greater antibody rise following feeding of 1.0cc of *trivalent oral poliomyelitis vaccine ($10^{7.8}$ TCD_{50} of each type of virus per dose)*



DISCUSSION

From the above results it is at once apparent that a much better neutralizing antibody response was obtained in individuals fed the 2.0 ml dose of trivalent poliovirus vaccine than in those receiving the 1.0 ml. dose. While the results obtained with the 0.5 ml dose look good it must be remembered that the number of people involved is quite small. It is important that these highly favorable results were obtained primarily in adults. We believe that this should be noted because the study carried out in Minnesota in 1958 clearly showed that children could be more readily immunized with the oral vaccine than could adults.[12] That is one of the reasons why the decision was made to use primarily adults in carrying out the study with the trivalent vaccine reported here.

It is obvious, of course, that from the standpoint of ease of administration and overall cost, a trivalent oral vaccine has many advantages to offer over the procedure of feeding the three virus strains separately. There is no doubt that a trivalent oral vaccine would be of great advantage to public health workers who ordinarily have limited time and funds with which to carry out mass immunization programs.

The percentage of persons responding to the trivalent vaccine appeared to be highest for Types 3 and 1 and somewhat less for Type 2 but it is of interest to note that the Type 2 component produces a highly satisfactory booster effect in those persons already having low or medium levels of Type 2 antibody. However from the frequency of the serotypes of virus seen in the disease as it is encountered under natural conditions, a lesser response to Type 2 would represent the least degree of risk. All knowledge to date, of course, indicates that the most important type to protect against is Type I, followed probably by Types 3 and 2 in that order.

Chart 5 Poliovirus Type 2 Antibody Response of 188 Persons Fed 2 0 cc, Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD_{50} of Each Type per Dose)

Antibody Titers												Total Number Fed	No With 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024		
<1 4	9	5	6	4	2	2	7	3	2	1	1	42	33
1 4							1	1	1			3	3
1 8			1		4	1		3	3	1	2	15	14
1 16			1		1		1	1	3		1	8	6
1 32					3	1	3	3	2	2		14	10
1 64						1	4		1	1	3	10	5
1 128						1	11	4	6		7	29	13
1 256								3	3	1	5	12	6
1 512									6	3	14	23	14
1 1024									3	2	10	15	
>1 1024										3	14	17	
Totals	9	5	8	4	10	6	27	18	30	14	57	188	101

Chart 4. Poliovirus Type 1 Antibody Response of 188 Persons Fed 2 0 cc Trivalent Oral Poliomyelitis Vaccinf ($10^{6\ 1}$ TCD_{60} of Each Type per Dose)

Pre-Feeding	Antibody Titers — Post-Feeding <1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024	Total Number Fed	No With 4-Fold or Greater Response
<1 4	2	2	9	5	10	5	12	1	7			53	51
1 4							1		1			2	2
1 8			2	3	1		2	2	2			12	7
1 16					1			2		1		4	3
1 32					2	5	6	3	4	1		21	14
1 64						1	5	2	1	1	3	13	7
1 128					1	2	10	9	10	2	2	36	14
1 256								5	5	1	2	13	3
1 512								2	8	3	5	18	5
1 1024									1	7	4	12	
>1 1024											4	4	
Totals	2	2	11	8	15	13	36	26	39	16	20	188	106

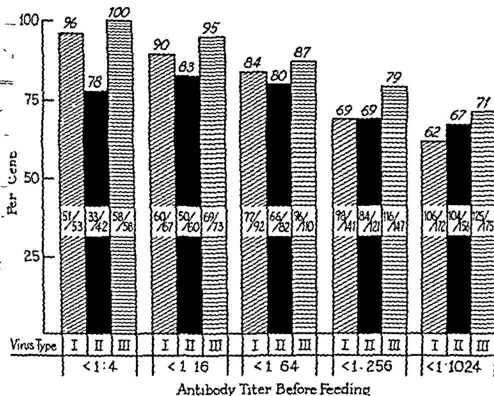
Chart 5 Poliovirus Type 2 Antibody Response of 188 Persons Fed 2.0 cc Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD$_{50}$ of Each Type per Dose)

	Antibody Titers												
Pre-Feeding	Post-Feeding											Total Number Fed	No With 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	9	5	6	4	2	2	7	3	2	1	1	42	33
1:4							1	1	1			3	3
1:8			1		4	1		3	3	1	2	15	11
1:16			1		1		1	1	3		1	8	6
1:32					3	1	3	3	2	2		14	10
1:64						1	4		1	1	3	10	5
1:128						1	11	4	6		7	29	13
1:256								3	3	1	5	12	6
1:512									6	3	14	23	14
1:1024									3	2	10	15	
>1:1024										3	11	17	
Totals	9	5	8	4	10	6	27	18	30	14	57	188	104

Chart 6 Poliovirus Type 3 Antibody Response of 188 Persons Fed 2.0 cc Trivalent Oral Poliomyelitis Vaccine
($10^{6.1}$ TCD_{50} of Each Type per Dose)

Antibody Titers												Total Number Fed	No With 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4		2	8	13	17	5	6	3	2	1	1	58	58
1:4			1									1	0
1:8			1	2	5		2	1	3			14	11
1:16				1	1	2	2	3	4			13	11
1:32					3	5	5		4	4	3	24	10
1:64					1	2	5	1	3		3	15	7
1:128							6	2	10	1	3	22	14
1:256								1	4		4	11	1
1:512									8	1	5	17	5
1:1024									1	3	2	6	
>1:1024										1	6	7	
Totals	0	2	10	16	27	14	26	13	39	14	27	188	126

FIG 2 Percentage of persons with fourfold or greater antibody rise following feeding of 2.0 cc of trivalent oral poliomyelitis vaccine ($10^{6.1}$ TCD₅₀ of each type of virus per dose)



American Cyanamid Company or their immediate families and all resided in a semi urban area within a 15 mile radius of Pearl River N Y

Good neutralizing antibody responses, as judged by primary conversions or a titer increase of at least 4-fold were elicited when each of the three types of virus was present in the mixture at concentrations of $10^{5.5}$ (300,000), $10^{5.8}$ (600,000) and $10^{6.1}$ (1 200 000) TCD₅₀. The most favorable results however were obtained in the group of 188 persons fed the largest dose, namely $10^{6.1}$ (1 200,000) TCD₅₀ of each of the three types of poliovirus in 2 0 ml of vaccine. Prior to vaccination with the single, oral dose of vaccine this group of 188 persons included 11 triple negatives 35 double negatives and 50 single negatives. Following vaccination, no double or triple negatives and only 11 single negatives remained. The conversion rate, therefore, was 93%. Two persons were left without antibodies to Type 1, nine remained negative to Type 2 and none was left without antibody to Type 3

These results indicate that it is both feasible and practicable to immunize against all three types of poliovirus by a single feeding of a trivalent, oral, poliovirus vaccine. The advantages of this type vaccine are discussed

ADDENDUM

Since this paper was prepared, serologic studies have been completed on another 286 persons fed a single dose of 2 0 ml. oral trivalent poliomyelitis vaccine, bringing the total to 474. Charts 7, 8 and 9 show their antibody response before and after feeding

A summary of antibody conversion effect as well as booster response of the 474 persons fed 2 0 ml. of vaccine is shown in Fig 3. Of those individuals with antibody titers originally <1 4

Chart 7 Poliovirus Type 1 Antibody Response of 474 Persons Fed 2 0 ml. of Trivalent Oral Poliomyelitis Vaccine (1,200,000 TCD_{50} Each Type per Dose)

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding											Total Number Fed	No With 4-Fold or Greater Response
	<1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024		
<1·4	9	5	20	13	33	15	28	7	15		1	146	137
1 4		1	1		3		1	1	1			8	6
1 8			6	4	4		3	4	5			26	16
1 16					5			2	2		1	10	5
1 32				1	10	13	8	4	9	3	2	50	26
1 64					2	6	9	4	5	1	6	33	16
1 128					1	5	29	17	24	3	3	82	30
1 256						1	1	18	17	2	4	43	6
1 512								4	32	9	6	51	6
1 1024									2	10	6	18	—
>1.1024											7	7	—
Totals	9	6	27	18	58	40	79	61	112	28	36	474	248

CHART 8 POLIOVIRUS TYPE 2 ANTIBODY RESPONSE OF 474 PERSONS FED 2 0 ML OF TRIVALENT ORAL POLIOMYELITIS VACCINE (1,200,000 TCD_{50} EACH TYPE PER DOSE)

	DISTRIBUTION OF ANTIBODY TITERS												
PRE-FEEDING	POST-FEEDING											TOTAL NUMBER FED	NO WITH 4-FOLD OR GREATER RESPONSE
	<1 4	1 4	1 8	1 16	1 32	1 64	1 128	1 256	1 512	1 1024	>1 1024		
<1 4	31	10	15	10	11	7	14	4	4	1	2	109	78
1 4					3	2	2	2	1			10	10
1 8			1	3	4	3	1	8	5	1	4	30	26
1 16			1		1	4	3	1	5		1	16	14
1 32				1	9	7	8	4	6	3	2	40	23
1 64						4	12	2	4	3	6	31	15
1 128						1	25	9	24	4	10	73	38
1 256							1	9	13	4	11	38	15
1 512								2	30	7	25	64	25
1 1024									5	6	17	28	—
>1 1024									1	4	30	35	—
Totals	31	10	17	11	28	28	66	41	98	33	108	474	244

Chart 9 Poliovirus Type 3 Antibody Response of 474 Persons Fed 2.0 ml of Trivalent Oral Poliomyelitis Vaccine (1,200,000 TCD_{50} Each Type per Dose)

Pre-Feeding	Distribution of Antibody Titers: Post-Feeding											Total Number Fed	No With 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	9	7	23	19	37	13	16	7	3	2	1	137	128
1:4			1			2	1					4	3
1:8			5	5	9	1	4	4	9	1		38	28
1:16				2	2	2	8	3	8		3	28	24
1:32					8	6	17	3	13	9	5	61	47
1:64					1	9	10	3	9	2	3	37	17
1:128						3	22	5	26	2	6	64	34
1:256							2	8	8	2	9	29	11
1:512								3	27	6	12	48	12
1:1024									3	11	2	16	—
>1:1024										2	10	12	—
Totals	9	7	29	26	57	36	80	36	106	37	51	474	301

94% responded with antibodies to Type 1, 72% to Type 2 and 93% to Type 3. Likewise, of all individuals who showed pre-vaccination antibody titers of <1:16, 88% showed either a primary or booster effect to Type 1, 76% to Type 2 and 89% to Type 3. Of persons with original antibody titers of <1·64, 79% showed a primary or booster effect to Type 1, 74% to Type 2 and 85% to Type 3, and of those with antibody titers of <1 256 before vaccination, 67% showed a fourfold or greater antibody rise for Type 1, 66% for Type 2 and 76% for Type 3. In all persons who showed initial antibody titers of <1·1024, the titers rose in 55% for Type 1, 59% for Type 2 and 68% for Type 3.

Prior to vaccination, this group of 474 persons included 392 antibody negatives. Following vaccination, only 49 negatives remained giving an overall conversion rate of 88%.

ACKNOWLEDGMENTS

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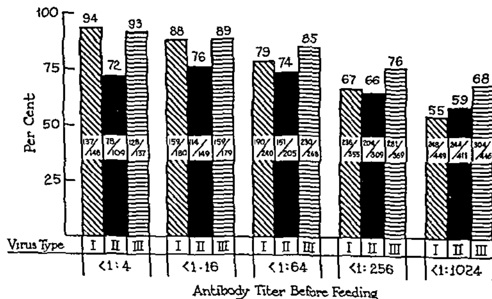
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Fig 3 Percentage of 474 persons with fourfold or greater antibody rise following feeding of 2.0cc of trivalent oral poliomyelitis vaccine (10⁵·¹ TCD₅₀ of each type of virus per dose)



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2. ANTIBODY RESPONSE IN ADULTS AND CHILDREN FOLLOWING SIMULTANEOUS ORAL ADMINISTRATION OF THREE TYPE STRAINS OF LIVE ATTENUATED POLIOMYELITIS VIRUS

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DR PREM *(presenting the paper)* An ideal immunization against poliomyelitis involves the use of a vaccine which closely mimics the protection resulting from natural infection without producing clinical disease. With this in mind, search has continued for attenuated live poliomyelitis viruses which can be simply and safely administered. The obvious advantages of such an approach do not require listing here.

Three such live attenuated poliomyelitis viruses have been made available for study to the Department of Obstetrics and Gynecology of the University of Minnesota Medical School. This report deals with a study designed to learn whether or not all three types of attenuated strains can be effectively administered simultaneously to children and adults.

MATERIALS AND METHODS

The vaccine The three strains of live attenuated poliomyelitis virus used in this study were the SM (Type 1), MEF_1 (Type 2), and the Fox (Type 3) strains developed under the direction of Dr Herald R Cox.* The attenuation histories of these strains and related monkey test results have been reported by Cabasso *et al*[1]

To simplify administration of the viruses a measured amount of each strain absorbed on granular gelatin was prepared by the manufacturer in a hard gelatin capsule. A different colored capsule was used for each strain. The amount of virus in each capsule was computed as $10^{4.9}$ TCD_{50},† for Type 1, $10^{5.1}$ TCD_{50} for Type 2, and $10^{5.3}$ TCD_{50} for Type 3.

Administration of the vaccine Because previous experience with the Type 2 strain indicated it to be antigenically inferior in the dose used to the Type 1 and Type 3 strains,[2] all participants in this study received two capsules or a double dose of this strain. In addition, each of the adults and twelve of the children received one capsule of each the Type 1 and Type 3 strains. The other nine children received two capsules of each of the three strains.

With one exception the adults and older children swallowed the capsules intact. One capsule was given after another and followed by a drink of water. One person preferred to chew each capsule before swallowing. The viruses were removed from the capsule, spoon-fed to the younger children and followed by water. None complained of a disagreeable taste. All vaccinations were conducted personally by one of the authors.

The first vaccine was fed on March 13, 1958 and the last on August 4, 1958.

Participants The adults who participated in the study were 27 pregnant women registered for private or clinic obstetric care at the University of Minnesota Hospitals and their husbands. All volunteered for the study after its purpose was outlined to them. All adult males participating were University of Minnesota students or staff members. Several of the adults and one child had participated in previous poliomyelitis vaccine studies by the authors.[2, 3] The average age of the adults was 25.4 years with a range of 19 to 34 years. Three of the women were in the first, fourteen in the second, and ten in the third trimester of pregnancy.

There were 21 children in this study. Twelve were the children of eight of the couples in the study. The other nine children were sons and daughters of staff members in the Department

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† TCD_{50}=tissue culture $doses_{50}$.

of Obstetrics and Gynecology All children were in the age range of seven months to nine years.

The number of Salk vaccine injections received prior to the beginning of each study by each of the 70 participants included in the final tabulation *is shown in Table 1 These figures* show that husbands in the families studied are more negligent about receiving Salk vaccine Twice as many husbands as wives had failed to receive at least a single Salk vaccine prior to participating in this study. Children had received more such vaccine than the adults Only one of 19 children had not had at least one injection of Salk vaccine prior to the study

Every participant agreed not to receive further Salk vaccine injections during the time period of this study

TABLE 1 SALK VACCINE INJECTIONS PRIOR TO PARTICIPATION IN THIS STUDY

	Number of Salk Vaccine Injections					
	0	*1*	*2*	*3*	*4*	*Totals*
Pregnant women	6	6	7	6	2	27
Husbands	12	2	4	6	0	24*
Children	1	1	4	13	0	19*

* Only those with paired pre- and post-vaccination blood samples included

Laboratory At the time of vaccination 5-10 cc of whole blood was drawn by venipuncture and placed in a sterile tube without anticoagulant A second blood sample was obtained from 65 of the participants 4-5 weeks after vaccination Seven of these blood samples hemolyzed and were replaced by specimens drawn 7-14 weeks after vaccination One post-vaccination specimen was drawn at three weeks and four others at six weeks

All antibody determinations were done by the *Division of Medical* Laboratories, Minnesota State Board of Health * The technique used to determine antibody titers is reported in detail elsewhere [4] and will not be repeated here By this technique a 16 fold (two tube) increase in antibody titer is significant. Titers less then 1:4 are considered unmeasurable.

* Director of Medical Laboratories, Henry Bauer, Ph D

SYMPTOMS

Close observation of the family groups for adverse reactions to the vaccine was accomplished through the pregnant mother who in each case was making regular prenatal visits The nine children of family groups in which the mother was not pregnant were under continuous observation for untoward reactions by their physician fathers with whom the authors had daily contact

One pregnant adult reported epigastric discomfort, nausea, vomiting, and low grade fever within 24 hours after feeding It is doubtful that these symptoms were related to the vaccine since they were identical with those experienced by several unvaccinated people with whom she worked No other signs or symptoms of illness were observed or reported All children in this study, including the nine who received double doses of Types 1 and 3, tolerated the vaccine as well as the adults

RESULTS

The results reported here are from the 27 pregnant women, 24 husbands, and 19 children for whom paired blood samples (before and after vaccination) were available for antibody titer comparisons Although three husbands and two children were fed vaccine, they were eliminated from the study because one or both blood samples were unsatisfactory for testing

Adults Nine (33%) of the 27 pregnant women responded to vaccination with a 16-fold increase in antibody titer to Type 1, five (19%) to Type 2, and four (15%) to Type 3 poliomyelitis virus Three (12%) of 24 husbands responded with a 16-fold rise in antibody level to Type 1; two (8%) to Type 2 and seven *(28%) to Type 3 Because these two groups* were too small to compare with each other statistically, the results were combined and are shown by correlation squares in Table 2 Of the total of 51 adults, 12 or *24% responded* with a significant antibody rise to Type 1, seven or 14% to Type 2, and eleven or 22% to Type 3

These results are compared in Table 3 with those obtained in a similar group of adults in a study by Barr *et al* [4] in which the same doses and strains of live attenuated virus were fed one strain at a time at three-week intervals in the order Type 2, Type 1 and Type 3

TABLE 2 ANTIBODY TITER RESPONSE AMONG 51 ADULTS AFTER SIMULTANEOUS FEEDING OF THREE TYPE STRAINS OF LIVE ATTENUATED POLIOMYELITIS VIRUS
(All Figures Above Heavy Line Indicate Number with 16 Fold or Greater Rise in Titer)

Pre-Vaccination Titer	Type 1						Type 2						Type 3					
1024					1	3				1	2	6		1	1		3	3
256		1		1	2	2	1			2	1	2	2	1	1	2	1	2
64	6		4	4			2		2	4	2		2		2	1	1	
16	5	2	1		3		3		1	1	1		3	4	2	2		
4	5	1		1			4	1	2				7		1			
<4	9						13						9					
	<4	4	16	64	256	1024	<4	4	16	64	256	1024	<4	4	16	64	256	1024

Pre-Vaccination Titer

TABLE 3 COMPARISON OF SIGNIFICANT ANTIBODY TITER RISES AMONG ADULTS WHEN IDENTICAL STRAINS AND DOSES OF LIVE ATTENUATED POLIOMYELITIS VIRUS ARE ADMINISTERED ORALLY BY TWO DIFFERENT TECHNIQUES

Feeding Technique	Total Vaccinated	Number and Percent with 16-Fold or Greater Rise in Antibody Titer		
		Type 1	Type 2	Type 3
Each strain at intervals of three weeks (after Barr[4])	282*	24 (8.5%)	44 (15.6%)	39 (14.2%)
All strains simultaneously	51*	12 (24%)	7 (14%)	11 (22%)

* Virus doses fed this group as follows SM (type 1) $10^{4.9}$ TCD_{50}, MEF_1 (type 2) $10^{5.4}$ TCD_{50}, Fox (type 3) $10^{5.2}$ TCD_{50}

The results as measured by significant antibody response in this study can be compared to those obtained by Barr since the serological testing for both studies were done in the same laboratory, by the same personnel, and by the same technique. This comparison in Table 3 indicates the results are at least as good when all three strains are given in the same dose simultaneously as when given singly at three-week intervals

Children Although different doses of virus were given to two groups of children in this study, the results in these groups could not be compared because of the small numbers Therefore, the antibody responses of the children were grouped together in correlation squares and shown in Table 4. Of the 19 children, 14 (74%) responded to vaccination with a 16-fold or greater rise to Type 1, eight (42%) to Type 2, and 16 (81%) to Type 3. Although no child had an unmeasurable titer to Types 1 or 3 after vaccination, two children continued with unmeasurable titers to Type 2

These results are compared in Table 5 with those reported by Barr *et al* [4] in which the same doses and strains of live attenuated virus were fed one strain at a time at three-week intervals Although ten of the children in this study received the same doses of virus as those used by Barr, nine received double doses of Types 1

TABLE 4. ANTIBODY TITER RESPONSE AMONG 19 CHILDREN AFTER SIMULTANEOUS FEEDING OF THREE TYPE STRAINS OF LIVE ATTENUATED POLIOMYELITIS VIRUS
(All Figures Above Heavy Line Indicate Number with 16-Fold or Greater Rise in Titer)

Post-Vaccination Titer	Type 1						Type 2						Type 3					
1024	5	1					1	1	1		1		2			1		
256	5				2		3	1	1	1			2	1			1	1
64	1	1					1		3				4					
16	1							1	1				6					
4	3						1		1				1					
<4							2											
Pre-Vaccination Titer	<4	4	16	64	256	1024	<4	4	16	64	256	1024	<4	4	16	64	256	1024

TABLE 5 COMPARISON OF SIGNIFICANT ANTIBODY TITER RISES AMONG CHILDREN AFTER IDENTICAL STRAINS OF LIVE ATTENUATED POLIOMYELITIS VIRUS ARE ADMINISTERED ORALLY BY TWO DIFFERENT TECHNIQUES

Feeding Technique	Total Vaccinated	Number and Percent with 16-Fold or Greater Rise in Antibody Titer		
		Type 1	Type 2	Type 3
Each strain at intervals of three weeks (after Barr[4])	255*	158 (61 9%)	146 (57 2%)	161 (63 1%)
All strains simultaneously	10* } 19 9**	8 } (74%) 6	2 } (42%) 6	10 } (84%) 6

* Virus doses fed to this group as follows SM (type 1) $10^{4.9}$ TCD_{50}, MEF_1 (type 2) $10^{5.4}$ TCD_{50}, Fox (type 3) $10^{5.5}$ TCD_{50}
** Virus doses fed to this group as follows SM (type 1) $10^{5.8}$ TCD_{50}, MEF_1 (type 2) $10^{5.4}$ TCD_{50}, Fox (type 3) $10^{5.6}$ TCD_{50}

and 3 The results of these two different doses are show separately in the table Although the group fed three type *strains simultaneously* is small, it is obvious that the results are not much different from the group in which these strains were fed singly.

DISCUSSION

Although the number of individuals is small, it appears that the three type strains of attenuated poliomyelitis virus used in this study can be administered simultaneously in fairly large *doses to adults and children alike without clinical* evidence of active infection.

The symptoms reported by one pregnant woman following vaccination were related, in all probability, more to epidemic gastrointestinal upsets prevalant in the area at the time of feeding than to the ingestion of the viruses.

The response of the children in this study as

measured by antibody titer rise after vaccination is superior to the response of the adults This is the same conclusion reached by Barr et al[4] when these same strains were fed singly at three-week intervals (compare Tables 3 and 5). The reasons for the poorer response in adults have not been clarified by this study

This study shows better antibody titer responses to Type 1 and Type 3 strains than to the Type 2 strain for both adults and children Whether this poorer response is due to inherent inferior antigenicity of the Type 2 strain or to "interference phenomena" observed by Koprowski[5] and Sabin[6] when more than one type strain is fed simultaneously is not known

Barr et al[4] found the Type 2 strain, when fed in the same dose as used in this study as potent an antigen as the Type 1 and Type 3 strains In his study, however the Type 2 strain was fed first and followed by the Type 1 and Type 3 strains at three-week intervals

Whether the Type 2 strain is a poorer antigen or whether its poorer showing in this study is due to failure to avoid "interference phenomena" is unimportant since this study appears to establish that simultaneous feedings of the Types 1, 2, and 3 strains can result in antibody responses as good or better than the same doses of the three strains given separately at three-week intervals

Of the 27 pregnant women who received live attenuated poliomyelitis virus in this study 26 were delivered at or near term of normal infants The other infant was very premature (1200 grams) and did not survive At autopsy this child was anatomically normal There was no evidence that this event was related to the administration of vaccine to the mother

CONCLUSIONS

The type strains of live attenuated poliomyelitis virus in the dosages used in this study can be simultaneously administered by mouth to children and adults, including pregnant women, without untoward reaction

These strains produced significant rises in antibody titer in a large percentage of the children in this study when given simultaneously by the oral route The antibody responses among adults were poorer

The antibody response to the Type 2 strain was inferior to the responses to Types 1 and 3 strains in both adults and children

Within the limits of this small series the antibody responses in both adults and children in this study were similar in degree to those obtained by others when the same strains in the same doses were administered at intervals of three weeks

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3. IMMUNOLOGIC RESPONSE OF INFANTS UNDER SIX MONTHS O AGE TO ORAL TRIVALENT POLIOMYELITIS VACCINE

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DR PREM *(presenting the paper)* This second report is just a preliminary one and summarizes to date the results of young-infant vaccination Perhaps it would have been wise not to report these results at this time because of the small numbers of infants studied thus far.

INTRODUCTION

It his been stated that the logical approach to the prevention of poliomyelitis is to produce a living virus vaccine that can be given safely in infancy under the protection derived either from passively transferred maternal antibodies or from gamma globulin[1, 2]

The development of strains of live attenuated poliomyelitis virus during the past decade has made the approach to polio protection through the infant very attractive Some of these strains when given individually by mouth or in a series with one strain at a time have produced active intestinal infections and significant antibody responses in infants without apparent ill effects[3, 4]

Recently a trivalent oral live attenuated virus vaccine has been developed It is expected that this vaccine, when it is fed once in proper dosage, will produce an active intestinal infection that will result in durable protection to all three types of poliomyelitis This vaccine was made available for study to the Department of Obstetrics and Gynecology of the University of Minnesota Medical School by Dr Herald R Cox.

This report deals with a study of this vaccine that was designed to:

(1) Determine the safety of this vaccine when fed to young infants

(2) Observe the antibody response of the newborn to this vaccine and compare it with the response among infants between three and s months of age

(3) Compare the immunologic responses the vaccine of breast and bottle fed infants

METHODS AND MATERIALS

Participants—Infants of six months of age less who had been born to mothers who receive obstetric care and were delivered at Universi of Minnesota Hospitals participated in th study The parents of these infants were st dents or staff members at the University Minnesota who volunteered their children f the study when the nature of the vaccine ar the purpose of the study was explained to ther Many of the mothers and some of the fathe had participated in other studies of poli myelitis vaccine by the authors Thirty thr mothers of infants studied were private and s were clinic patients. The father or mother twelve of the infants was a physician Tl mothers of at least five others were registere nurses No infant in this study had previous received Salk vaccine.

For this study the infants were divided in two groups One group contained only ne borns The other was made up of infants fro *about 3 to 6 months of age.*

Vaccine—The strains of live attenuated poli myelitis virus in the trivalent vaccine used i this study were the SM (Type 1), MEF$_1$ (Typ 2), and Fox (Type 3) strains developed b Lederle Laboratories under the direction of D Cox The vaccine was prepared as a cherr flavored liquid suspension with each cubi centimeter containing approximately $10^{5.6 \text{ to } 6}$ TCD_{50}* of each strain The three different lot of vaccine used were 7-1231-800, 7-1231 801 and a mixture of equal parts of lots 7-1231-121

* Tissue culture doses$_{50}$

7 1232 216 and 7-1233 318. All vaccine was stored at constant temperature of 4° C. until used

Administration of vaccine.—Each of the newborn infants was fed one or two drops of the vaccine at a time with a calibrated dropper until 0.5 or 1 0 cc. was given The vaccine was dropped directly into the infant's mouth and followed by feeding at the breast or bottle

Twenty newborn infants received vaccine just prior to the last feeding before discharge from the newborn nursery. Four were brought back to the outpatient department for vaccination Of these infants, nineteen were four to seven days old, two were ten, and one each was 11, 16 and 24 days old at time of vaccination

Each of the three lots of vaccine was given to a part of the newborn group. Thirteeen infants received one and eleven received one-half cubic centimeter of vaccine

Of the 15 older infants fed the vaccine, one was two and one half months, eight were three months, one was four months, and five were six months of age at the time of vaccination

Two lots only were used to vaccinate the older infants None of these infants was given more than one half cubic centimeter

Laboratory—Cord blood was collected at delivery from most of the infants in the newborn group for determination of maternal antibodies passively transferred from the mother during intrauterine life. All other pre-vaccination and post vaccination blood samples were obtained by external or internal jugular venipuncture. For 26 infants the interval between vaccination and post-vaccination blood sample was four to six weeks; for seven others, seven to nine weeks, and for one each three, ten and fifteen weeks All blood samples were aseptically collected without an anticoagulant After clot retraction and centrifugation, the serum was transferred by sterile pipette to a sterile tube and frozen When a sufficient number of pairs of pre- and post-vaccination sera accumulated they were sent by air express to the Viral and Rickettsial Research Sections of Lederle Laboratories, Pearl River, New York, for serological testing

The method of antibody determination used was the pH or color test according to the procedure of Salk and Youngner[5] All sera were first inactivated for 30 minutes at 56°C in a constant temperature water bath. The serum samples were then prepared in four-fold dilutions in duplicate 1:4 through 1 1024 Approximately 100 to 300 TCD_{50} of the representative strains of virus were added to the respective serum dilutions and the mixtures held at room temperatures for three hours. Trypsinized monkey kidney tissue cell suspensions were added to each of the serum-virus mixtures and to appropiate controls The tubes were kept at constant temperature of 37°C. and read on the sixth or seventh day Antibody titers were calculated by the method of Reed and Muench[6]

By this technique a four fold rise in antibody titer is significant

Calculation of Antibody Titer Response to Vaccination in the Young Infant Because techniques for antibody titer determinations do not differentiate between the sources of antibody, it is necessary, if the effect of vaccination of the young infant is to be known, to be able to determine at any age the amount of circulating passively transferred maternal antibody that remains

A previous study[7] of poliomyelitis antibody transfer from mother to newborn infant and follow-up of the infant during the first year of life has shown that the antibody disappears from the newborn at a uniform rate with a half-life value of five weeks (37 days) This is shown graphically in Figure 1 This study also showed that the duration of persistence of antibody is directly

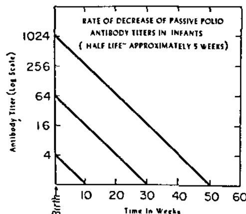


FIG 1 Rate of decrease of passively transmitted maternal antibodies in newborn infants[7]

related to the level of antibody titer at birth Infants with a cord blood titer of 1 to 1024 continue to have detectable titers until about one year of age. This knowledge permits calculation of the residual passively transferred antibody titer in the newborn at any age providing the antibody titer at birth is known

(1) *In the newborn*—The antibody response of the newborn to the vaccine in this study was determined by comparing the measured antibody titer in the post-vaccination blood to the expected residual antibody titer of maternal antibodies as calculated by the half-life date on the date the post-vaccination blood was drawn

To conveniently plot the antibody rise in correlation squares, the newborn was assumed to have one-half of the antibody titer present at birth from age 19 to 56 days (one half to one and one-half times the half-life) and one-fourth from age 57 to 94 days (one and one-half to two and one-half times the half-life). If the post-vaccination antibody titer was four times the calculated remaining maternal antibody level, the response to vaccination was considered significant

(2) *Infants age 3-6 months*—Among infants in this age group, the residual titer of passively transmitted maternal antibodies as calculated from half-life tables is about ¼ to 1/32 of the titer present at birth Since the antibody titer at birth was not known in most of the infants, the titer at the time of vaccination was used as a baseline and compared with the titer after vaccination Although this is not a completely valid comparison since passively transferred antibodies are still present and decreasing, any errors introduced are in a conservative direction and tend to worsen the results If the post-vaccination antibody titer was four times the pre-vaccination titer, the response to vaccination was considered significant.

SYMPTOMS

Despite the large number of physicians and nurses in daily contact with these infants not a single untoward reaction was seen or reported in any of the 39 infants fed The newborn infants tolerated all doses and lots of vaccine as well as the older infants.

RESULTS

Newborn infants—Paired pre- and post-vaccination blood samples were available for comparison of antibody titers in 23 of the newborn infa fed the vaccine. Because each mother in t series had received oral live attenuated po myelitis virus and/or Salk vaccine prior to during pregnancy, nearly all of these infa were born with measurable antibody titers to types of poliomyelitis virus One infant, ho ever, lacked measurable antibody to Type another to types 1 and 3 and four others Type 3 All of these except one infant with unmeasurable titer to Type 3 converted to positive titer after vaccination Of the to group, seven (30%) experienced a four fold greater rise to Type 1; one (4%) to Type and ten (43%) to Type 3. These results shown by correlation squares in Table 1. T

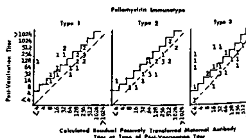


TABLE 1 ANTIBODY RESPONSE OF NEWBORN INFA FED TRIVALENT ORAL POLIOMYELITIS VACCINE Co PARISON OF CALCULATED PASSIVELY TRANSFERRED M TERNAL ANTIBODIES WITH POST VACCINATION TI (All Figures Above Stairs Are Four fold or Grea Responses)

correlation squares show that the significa rises in antibody titer are mainly confined those newborns who have a calculated residu titer of maternal antibodies of 1.32 or less only these low titers are considered, the numb of infants responding to vaccination with si nificant antibody titer rise becomes 7 to 9 (78% to Type 1, 1 to 6 (17%) to Type 2; and 9 15 (60%) to Type 3 Since there was only o four-fold or greater rise following vaccinati when the calculated residual maternal antibo titer is 1:64 or greater, it appears that a hig initial antibody titer interferes with or masks significant early antibody response to a challeng at birth with live attenuated virus In this sma group of infants no important difference can b detected between the lots and doses of vaccin used

Infants 3 to 6 months of age—Few infants at this age had antibody titers at the time of vaccination greater than 1:32 except for Type 2 Four of the five infants with high antibody titers to Type 2 had mothers with titers of 1 512 or higher at delivery A fourth had an unmeasurable titer to Type 2 at three months of age but the blood taken at vaccination showed a titer of 1:1024 This high titer presumably was the result of an active immunization experience to a wild Type 2 poliovirus.

The correlation squares in Table 2 show an eight fold or greater rise in 10 (80%) to Type 1, a significant (four fold) rise in only 1 (8%) to Type 2; and 12 (92%) to Type 3 The results except for Type 2 are excellent considering the small dose of vaccine used (0.5 cc).

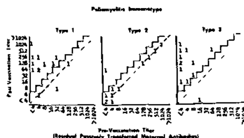


TABLE 2 ANTIBODY RESPONSE OF INFANTS AGE 3-6 MONTHS TO TRIVALENT ORAL POLIOMYELITIS VACCINE (All Figures Above Stairs Are Four fold or Greater Responses)

The results from vaccination of both the newborn and the older infants with antibody titers of 1 64 or less at the time of vaccination (1 32 five weeks after vaccination) are compared in Table 3 (Compare also Tables 1 and 2) This indicates that the response among both age groups is very similar for this range of antibody titers except for Type 3 which in the newborn may be slightly inferior I doubt if these groups are large enough to compare statistically

Antibody Response Related to Type of Feeding—Ten of the fourteen breast fed newborn infants and four of nine bottle-fed infants had four-fold or greater rises to one or two types Within the limit of the small groups studied, these results suggest that newborn infants respond as well, if not better, to challenge with live attenuated poliomyelitis virus if they are breast-fed

TABLE 3 COMPARISON OF RESPONSE TO TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE AMONG INFANTS BELOW AGE OF SIX MONTHS WITH ANTIBODIES OF 1 64 OR LESS AT TIME OF FEEDING

	Number and Percent with Four-Fold or Greater Response	
Immunotype	Newborn Infants	Infants Aged 3–6 Months
T_1	8/9 (89%)	10/12 (83%)
T_2	1/6 (17%)	1/8 (12%)
T_3	9/15 (60%)	11/12 (92%)

DISCUSSION

From these results it appears that trivalent oral poliomyelitis vaccine evokes a prompt significant antibody response to Types 1 and 3 when fed to infants who have an absent or low titer of passively transferred maternal antibodies. The newborns in this study who failed to respond to vaccination with a significant rise in titer were those who had higher levels of passively transferred antibodies at birth Osborn [5] observed that a high pre-vaccination titer of antibodies in the infant is liable to depress the rate of active immunization The findings of this study tend to confirm this This does not mean, however, that the vaccination of these infants was unsuccessful. Koprowski [3] has shown that strains of live attenuated poliomyelitis virus can develop an intestinal infection in a newborn infant despite high passively obtained antibody titers If this intestinal infection persists for a long period of time, ultimately antibody rise or stabilization occurs as the passively transferred maternal antibodies disappear This knowledge makes it necessary to obtain antibody titers at a longer time than one month after vaccination if the ultimate response to vaccination of the newborn is to be adequately evaluated

The response to trivalent oral poliomyelitis vaccine of the infant who is three to six months of age, is immediate and excellent to Types 1 and 3 At this age the infant usually has an absent or low residual titer of passively trans-

ferred maternal antibodies which does not mask or delay the response to active immunization

Among both the newborns and the older infants studied, the response to Type 2 strain is poorer than to the Type 1 and Type 3 strains Other studies of these same strains have confirmed this [4, 9] This poorer response may be due to inferior antigenicity, inadequate inoculation dosage, or interference phenomena reported by Sabin [10] and Koprowski [11] The last of these possibilities is the least likely because another study [4] has shown poorer response to the Type 2 strain also when fed singly

It appears from these data that the immediate response to this vaccine in newborns with a low passively transferred maternal antibody titer is as good as that in infants three to six months of age To determine whether this statement is true or not for newborns with higher antibody levels at birth, additional follow-up is necessary until the passively transferred maternal antibodies have disappeared In infants born with high initial antibody titers this may take nearly one year [7]

Although human milk contains antibodies against poliomyelitis, Sabin [2] has shown that the cynomolgus monkey received no beneficial protective effect when fed human milk before, during, and after challenges with a live virulent virus Although the number of infants in this study is small it appears that human milk does not interfere with the antibody response of the newborn to oral poliomyelitis vaccine In fact, the percent of significant antibody rises among breast-fed babies is actually higher than among bottle-fed babies A study with a larger series of newborn infants is underway to determine if this is a valid observation

CONCLUSIONS

Thirty-nine infants younger than six months of age have been fed trivalent vaccine containing large quantities of the Type 1, Type 2, and Type 3 strains of live attenuated poliomyelitis virus without untoward effect

The response to vaccination as measured by antibody rise among infants from three to six months of age and among newborns with low titers of passively transferred maternal antibodies at birth is excellent to Types 1 and 3 but poorer to Type 2

The response to vaccination of the newborn who has a high antibody titer at birth cannot be accurately determined until these passively received antibodies have disappeared

Breast feeding does not appear from this study to unfavorably influence the immunologic response of the newborn to the trivalent oral poliomyelitis vaccine used.

ADDENDUM Since this paper was written over 50 more newborn infants have been given 1.0 cc and 2.0 cc of trivalent oral poliomyelitis vaccine without a single untoward reaction seen or reported

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4. IMMUNOLOGIC RESPONSE OF PREGNANT WOMEN TO ORAL TRIVALENT POLIOMYELITIS VACCINE *

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DR. PREM *(presenting the paper)*: It seems well established that the pregnant woman is more susceptible than others to poliomyelitis. During the Minnesota epidemic in 1946, the attack rate in pregnant women was calculated at 74/100,000 as compared to 47/100,000 among non-pregnant women[1] A study of poliomyelitis in Massachusetts has reported a poliomyelitis attack rate during pregnancy which was three times that expected[2]

Siegel and Greenberg[3] as well as Anderson[1] have shown the pregnant women to be most susceptible to poliomyelitis between the third and fifth months of gestation The rate increase during this time of pregnancy was responsible for the overall increase in incidence reported during pregnancy by Weinstein[2] Rindge[4] has also shown the second trimester attack rate to be higher than expected from chance alone

Weinstein[2] has suggested that sex hormones may influence the susceptibility to poliomyelitis In a high per cent of the women he observed, there seemed to be a relationship between the onset of poliomyelitis and that part of the menstrual cycle surrounding the time of menstrual flow He also suggested that the increased incidence of this disease in pregnancy may be secondary to the effect of hormones other than the sex hormones since the activity of the thyroid, adrenal and other endocrine glands may be markedly altered during pregnancy Aronson[5] has shown that cortisone increases the vulnerability to paralysis of muscles of the animal with experimental poliomyelitis.

The fetal loss in mothers who contract poliomyelitis during pregnancy has been calculated by Anderson[1] as 23%. Although Rindge[4] reported a fetal loss rate of 27 8%, some of these losses were due to obstetrical complications unrelated to poliomyelitis Horn[6] has stated that even mild non-paralytic attacks of poliomyelitis may increase the abortion rate.

Because of these relationships the response of the pregnant woman to live attenuated poliomyelitis virus deserves detailed study. The recent development of safe live attenuated type strains of poliomyelitis virus has made a study of this kind possible

Three live attenuated type strains of poliomyelitis virus in the form of an oral trivalent vaccine were made available for study to the Department of Obstetrics and Gynecology of the University of Minnesota by Dr Herald R. Cox

This report deals with a study of this vaccine that was designed to:

(1) Determine the safety of this vaccine when fed to pregnant women in all trimesters of pregnancy

(2) Observe the antibody response of the pregnant woman when given a single dose of this trivalent vaccine

(3) Compare the immunologic response to oral vaccine of pregnant women with naturally-occurring antibodies to a similar group who had received commercially produced Salk vaccine during pregnancy

(4) To compare the immunologic responses when the oral trivalent vaccine is fed to pregnant women in two separate doses.

MATERIALS AND METHODS

Participants.—Two hundred and twenty-three pregnant unwed girls receiving prenatal care at Booth Memorial Hospital (BMH) and Catholic Infants' Home (CIH) in St. Paul, Minnesota, participated in this study All of these girls reside at BMH or CIH during the last six weeks

* This report is part of a larger study partially financed by the Elizabeth Kenny Foundation and Lederle Laboratories through the Minnesota State Board of Health Lederle Laboratories provided all the vaccine used in this study and facilities and technicians for all laboratory determinations.

of their pregnancy. Although some live there during most of their pregnancy, most live in private homes or with parents during the earlier months of gestation All make prenatal visits to the clinic at the usual intervals The prenatal care at both institutions is conducted by the resident staff from the Department of Obstetrics and Gynecology of the University of Minnesota Hospitals. Nursing personnel are in continuous attendance at each institution

The ages, previous Salk experience, and trimester of pregnancy of these girls are shown in Table 1 128 girls were vaccinated at Booth Memorial Hospital and 95 at Catholic Infants' Home The average age for each group is approximately the same Fifty-four had not received Salk vaccine prior to participation in this study Among the 73 who had received one or two injections of Salk vaccine prior to partaking in this study were 36 girls who had received the vaccine for the first time after they reported for prenatal care This increases the number in this study who had not received Salk vaccine prior to the present pregnancy to 90 or 40 4% of the entire group Three were vaccinated with the oral vaccine during the first trimester of pregnancy, 61 during second trimester, and 158 during the third trimester

Vaccine—The vaccine used in this study consisted of equal amounts of three type strains of live attenuated poliomyelitis virus mixed into a cherry flavored liquid medium The viruses used in the vaccine were the SM (Type 1), MEF$_1$ (Type 2), and Fox (Type 3) strains developed at Lederle Laboratories under the direction of Dr Cox The attenuation history of these strains has been reported by Cabasso[7] Two lots of vaccine were used Lot #7 1238-801 was dispensed in 2 0 cc individual or 25 cc multiple dose vials with calibrated dropper Each 2 0 cc of the vaccine contained approximately $10^{9.1}$ TCD*$_{50}$ of each type strain Lot #7-1238 800 was dispensed in one 25 cc multiple dose vials Each 1 0 cc contained approximately $10^{7.8}$ TCD$_{50}$ of each type strain All vaccine was stored at a constant temperature of 4°C until used Extensive immunologic testing of lot #7-1238 801 in 1 0 and 2 0 cc doses has been done and results compiled by Cox[8]

Method of Administration.—One or two cubic centimeters of the vaccine was poured from the individual vial or squirted by dropper directly into the mouth of each participant A small quantity of water was taken immediately afterward by most of those vaccinated All feedings were at random either morning or afternoon with no set relationship to meals One of us (KAP) personally fed the vaccine and collected the blood samples from about one half of the participants All data reported in this study were collected from vaccinations administered between 28 January 1959 and 30 April 1959

Laboratory.—At the time of vaccination, ten cubic centimeters of whole blood was collected with sterile vacumatic tube by antecubital venipuncture Four weeks later a post vaccination blood specimen was obtained in the same manner Although this interval was achieved in 106 instances, thirty-three specimens were obtained for various reasons at two, three, or five to nine weeks following vaccination

After clot retraction at room temperature the specimens were centrifuged and the serum removed by sterile pipette, placed in sterile serum tube, frozen, and stored at −20° C Any blood specimens that did not have the serum separated immediately were refrigerated at 4° C until such time as this separation could be done When sufficient numbers of paired serum specimens accumulated they were shipped without refrigeration by air express to the Viral and Rickettsial Research Section of Lederle Laboratories, Pearl River, N Y for serological testing

The method of antibody determination used was the pH or color test according to the procedure of Salk and Youngner[9] All sera were first inactivated for 30 minutes at 56° C in a constant water bath The serum samples were then prepared in four-fold dilutions in duplicate, 1 4 through 1 1024 Approximately 100 to 300 TCD$_{50}$ of the representative strains of virus were added to the respective serum dilutions and the mixtures held at room temperatures for three hours Trypsinized monkey kidney tissue cell suspensions were added to each of the serum-virus mixtures, and to appropriate controls The tubes were kept at constant temperature of 37° C and read on the sixth or seventh day Antibody titers were calculated by the method of Reed and Muench[10] By this technique a four

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(2) Observe the antibody response of the pregnant woman when given a single dose of this trivalent vaccine

(3) Compare the immunologic response to oral vaccine of pregnant women with naturally occurring antibodies to a similar group who had received commercially produced Salk vaccine during pregnancy

(4) To compare the immunologic responses when the oral trivalent vaccine is fed to pregnant women in two separate doses

MATERIALS AND METHODS

Participants—Two-hundred and twenty-three pregnant unwed girls receiving prenatal care at Booth Memorial Hospital (BMH) and Catholic Infants' Home (CIH) in St. Paul, Minnesota, participated in this study. All of these girls reside at BMH or CIH during the last six weeks

* This report is part of a larger study partially financed by the Elizabeth Kenny Foundation and Lederle Laboratories through the Minnesota State Board of Health Lederle Laboratories provided all the vaccine used in this study and facilities and technicians for all laboratory determinations

TABLE 3 PERCENTAGE OF 139 PREGNANT WOMEN WHO ACHIEVED FOUR-FOLD AND TWO FOLD POSITIVE RESPONSES TO TRIVALENT ORAL POLIOMYELITIS VACCINE TABULATED ACCORDING TO DOSE AND LOT OF VACCINE

	Total Fed	Vaccine Dose	Vaccine Lot #	Antibodies, Type					
				Percent with Four-fold or > Increase			Percent with Two-fold or > Increase		
				T_1	T_2	T_3	T_1	T_2	T_3
BMH	32	1.0 cc*	−800	56.3	43.8	37.5	78.0	65.6	53.1
	23	1.0 cc*	−801	47.8	30.4	43.5	65.2	60.9	65.2
	25	2.0 cc†	−801	28.0	44.0	36.0	56.0	76.0	56.0
	80			45.0	40.0	38.8	67.5	67.5	57.5
CIH	59	2.0 cc†	−801	39.0	20.3	30.5	54.2	52.5	57.6
Totals	139			42.5	31.7	35.3	61.9	61.2	57.6

* $10^{\cdot 3}$ TCD_{50} each strain
† $10^{6.1}$ TCD_{50} each strain

The per cent of individuals who responded with a two-fold or greater and four fold or greater increase in titer are tabulated according to lot and dose of vaccine in Table 3. A four fold or greater rise in titer was present to Type 1, in 42.5%, Type 2, in 31.7%, and Type 3 in 35.3% of the *entire group*. *At least a two fold* rise in titer was observed in 61.9% to Type 1, 61.2% to Type 2, and 57.6% to Type 3. These latter data demonstrate a rather uniform antibody response to the three strains of virus used in the vaccine. Because Tables 2 and 3 give only the overall results they include many individuals who already had antibody titers of various levels to poliomyelitis because of natural immunity or previous administrations of Salk vaccine. To determine the real immunologic worth of the vaccine it is necessary to evaluate its effect among those who have undetectable antibodies before vaccination.

Of the 139 with complete serologic testing there were 37 (26.6%) who had an unmeasurable antibody titer to one, two or three poliomyelitis immunotypes before vaccination. These, together with their antibody response to trivalent oral vaccine, are tabulated in Table 4. After vaccination one girl continued to have an unmeasurable antibody titer to Type 2 and another to Type 3. No triple or double negatives remained after vaccination. Of the triple negatives before vaccination four had received no Salk vaccine and one three Salk injections. Of the double negatives two had received three Salk injections, two had received one, and the other *seven, none*.

A tabulation of unmeasurable antibody titers to the three poliomyelitis immunotypes according to the number of Salk vaccine injections prior to oral vaccination is shown in Table 5. As would be expected the greatest number and per cent of unmeasurable antibody titers to all three immunotypes appeared in the group that had not received Salk vaccine. After the first and second injections of Salk vaccine the number and per cent of unmeasurable immunotype antibody titers decreased. However, among those who have three or more Salk injections, there was a higher per cent of unmeasurable antibody titers for Types 2 and 3 than in those who had had one or two injections of Salk vaccine. Many of those who received three Salk injections had had them several years ago when the vaccine was first available and have had no booster dose since.

Of the total of 139 who were given oral trivalent vaccine and on whom data were com

TABLE 1 AGE, TRIMESTER OF PREGNANCY, AND PREVIOUS SALK IMMUNIZATION EXPERIENCE OF 223 PREGNANT GIRLS WHO RECEIVED TRIVALENT ORAL POLIOMYELITIS VACCINE

	Number of Pregnant Girls	Age		Trimester of Pregnancy			Number of Previous Salk Injections					
		Range	Average	1	2	3	0	1*	2**	3	4	?
Booth Memorial Hospital (BMH)	128	12–41	19 0	1	31	96	32	17	24	50	3	2
Catholic Infants' Home (CIH)	95	14–32	19 2	2	30	63	22	10	22	39	1	1

* 20 received this single Salk injection during present pregnancy
** 16 received these two Salk injections during present pregnancy

fold rise in antibody titer is significant Any titer of less than 1 4 is considered unmeasurable

SYMPTOMS

Although all of the participants were under direct or indirect observation through the maternity homes after vaccination, no adverse symptoms were reported to the nursing personnel on duty, the resident staff, or to the authors One girl with an allergic background who received the vaccine subsequent to the 223 reported here reported headache, nausea, vomiting, diarrhea and low grade temperature within 24 hours Since then she has taken another 2 0 cc of the vaccine without any symptoms She stated that she *had milder but identical reactions after* each of three injections of Salk vaccine

RESULTS

Pre vaccination and post-vaccination antibody titers have been completed on the sera of 139 of the 223 participants Many of the post-vaccination blood specimens have been too recently obtained to allow inclusion of the results in this paper. A few blood specimens were broken and some of the participants lost to follow-up because of delivery or discontinuance of care before the post vaccine blood specimen was obtained The percentage distribution of antibody titers by immunotype before and after vaccination is shown in Table 2 To make the data presented in Table 2 and subsequent similar tables more compact and allow easier comparison with other data later, only four-fold dilutions between 1·4 and 1·1024 are tabulated The two-fold dilutions of 1.8, 1.32, 1 128, and 1 512 are included in the next lower dilution (i e 1 8 included in 1 4; 1·32 in 1·16, etc) In this entire group 12 2 per cent had no measurable antibody titer to Type 1, 9 4 per cent to Type 2, and 19 7 per cent to Type 3 Note the conversion from lower titer (1.16 or less) to higher titer (1 64 or greater) for each immunotype Only two in the entire group continued to have an unmeasurable titer to a single immunotype after vaccination This is 1.4 per cent of the total of 139 After vaccination, all the negatives were converted to positive for Type 1 One failure was recorded for Type 2 and one for Type 3.

TABLE 2 PERCENTAGE DISTRIBUTION OF POLIOMYELITIS ANTIBODIES BY IMMUNOTYPE AND TITER AMONG 139 PREGNANT WOMEN BEFORE AND AFTER FEEDING ONE DOSE OF ORAL TRIVALENT VACCINE

Titer Level	Antibodies, Type: Before Vaccination			Antibodies, Type: After Vaccination		
	1	2	3	1	2	3
>1024	5 0	13 0	8 0	11 5	27 3	11 7
1024	8 6	12 2	7 3	10 8	15 2	8 0
256	33 8	33 1	31 4	49 6	37 4	42 3
64	27 3	22 3	14 6	23 0	11 5	19 7
16	10 8	9 4	13 1	4 4	5 0	13 1
4	2 2	0 7	5 0	0 7	2 9	4 4
<4	12 2	9 4	19 7	0 0	0 7	0 7

TABLE 5 RESPONSE TO TRIVALENT ORAL POLIOMYELITIS VACCINE AMONG 37 PREGNANT WOMEN WITH ONE OR MORE UNMEASURABLE (<1.4) PRE VACCINATION ANTIBODY TITERS AND CLASSIFIED ACCORDING TO IMMUNOTYPE AND NUMBER OF PREVIOUS SALK VACCINE INJECTIONS

Number of Previous Salk Injections	Number of Pregnant Women		*Antibodies, type* Before Vaccination: Number with Antibody Titer <1.4			After Vaccination: Number with Four-fold or > Increase			After Vaccination: Number with Two-fold or > Increase		
	Total	With 1 or More Antibody Titers <1.4	1	2	3	1	2	3	1	2	3
None	37	20	10	10	15	10	9	11	10	10	11
One	17	5	3	0	4	3	0	3	3	0	4
Two	28	4	2	0	2	2	0	2	2	0	2
Three	57	8	3	3	6	3	2	5	3	2	6
Totals	139	37	18	13	27	18	11	21	18	12	20

Number unmeasurable titers to all immunotypes 58
Number and per cent with four fold increase 53 (91%)
Number and per cent with two fold increase 56 (96.5%)

TABLE 4. DISTRIBUTION OF IMMUNOTYPE NEGATIVES AMONG 139 PREGNANT WOMEN BEFORE AND AFTER FEEDING OF TRIVALENT ORAL POLIOMYELITIS VACCINE

Numbers with Unmeasurable (<1:4) Antibody Titer

	Polio Immunotypes	BMH		CIH		Totals	
		Before	*After*	*Before*	*After*	*Before*	*After*
Triple negative	1–2–3	3	0	2	0	5	0
Double negative	1–2	1	0	1	0	2	0
	1–3	4	0	1	0	5	0
	2–3	4	0	0	0	4	0
Single negative	1	4	0	2	0	6	0
	2	1	0	1	1	2	1
	3	8	1	5	0	13	1
Total with one or more negative immunotypes		25	1	12	1	37	2
Total pregnant girls		80	80	59	59	139	139
Percent with one or more negative immunotypes		31.2%	1.3%	20.3%	1.7%	26.6%	1.4%

piled, there were 37 participants who showed 58 unmeasurable titers to the three immunotypes before vaccination. There were 27 unmeasurable antibody titers to Type 3, 18 to Type 1; and 13 to Type 2. Of these, 53 (91.4%) responded to oral vaccination with a four-fold or greater rise in antibody titer. When three others with a one tube rise in titer are added, the total positive antibody response to vaccination was 96.5%. Whether or not the participant had previously received Salk vaccine did not seem to influence the conversion response from unmeasurable to measurable. These numbers were too small to determine whether or not an anamnestic response occurred when the oral vaccine was given to those who had previously received Salk vaccine.

Thirty-seven (26.6%) of the 139 who have had antibody titers determined, had received no Salk vaccine prior to feeding of the trivalent oral vaccine. Among these were 20 who had negative antibody titers to one or more types. Ten were negative to Type 1, 10 to Type 2, and 15 to Type 3.

After vaccination 100 per cent of those who had a negative titer to Type 1 prior to vaccination responded with a four-fold or greater increase. Nine of the 10 with a negative titer to Type 2 had a four-fold or greater increase, and 14 of the 15 with a negative titer to Type 3 had a four-fold or greater increase after vaccination.

If only twofold increases were taken, there were 100 per cent responses to Type 1 and Type 2, and a 93 per cent response to Type 3 in this group with no previous Salk vaccinations.

Of the 17 who had received only one Salk injection (many of these had this injection just prior to the administration of the oral vaccine) five had unmeasurable antibody titers to one or more immunotypes. Although all had measurable titers to Type 2, three had unmeasurable titers to Type 1 and four to Type 3. This suggests that Type 2 response to Salk vaccine is

yet had an unmeasurable antibody titer before receiving oral vaccine. This was a failure to Type 2 in an individual who had received three doses of Salk vaccine The pre and post vaccination antibody titers of these 37 with no Salk experience are shown by immunotype in Table 6 The pre-vaccination antibodies presumably are naturally occurring and the result of subclinical infections with wild poliovirus. 40 5 per cent of these women had a pre-vaccination titer of 1 to 16 or less to Type 1, 37.8 per cent to Type 2, and 67.5 per cent to Type 3 The figures in this table show that the per cent of *those with low (1 16 or less) antibody titer to* Type 1 was reduced after oral vaccination from 40 to 8%, to Type 2, from 38 to 16%, and to Type 3, from 67 to 35% Only one individual of the 37 failed to convert an unmeasurable antibody titer to a measurable one This was a failure to Type 3 A similar table published by da Silva and the authors [11] comparing naturally-occurring antibody titers prior to vaccination with Salk vaccine among 186 pregnant women and after two injections of Salk vaccine among 136 pregnant women are shown in Table 7 A comparison of Tables 6 and 7 shows that fewer women *remain with unmeasurable anti*body titers after one dose of oral vaccine than following two doses of commercially-produced Salk vaccine In the Salk vaccinated group 11.2 per cent still had unmeasurable antibody titers to Type 1, 0 7 per cent to Type 2, and 10 3 to Type 3 However, in the group that received oral vaccine there were fewer titers of 1 1024 or greater for all three types than in the group that received Salk vaccine

Because oral vaccine lot #7-1238-800 is actually the same as #7 1238-801, the results obtained in the two groups who received 1 0 cubic centimeter of these lots were combined to compare with the results among the larger group of participants who received 2 0 cubic centimeters of vaccine lot #7-1238 801 All of the 55 participants who received the 1 0 cubic centimeter dose were patients at BMH Of the 84 who received the larger dose 59 were patients at CJH *and the remainder at BMH The bar* graph in Figure 1 compares the per cent of positive antibody titer responses (a two-fold or greater titer rise) for each of the two doses for each poliomyelitis immunotype At first glance it appears that the smaller dose produces as good or better a response for each immunotype However, if the responses among those with low pre-vaccination titers (1:32 or less) are separated it appears that the 2 0 cubic centimeter dose is superior although the numbers are small If those with high pre-vaccination titers (1 64 or greater) are separated, the Type I response to vaccination is much better for the 2 0 cubic centimeter dose, and the Type 2 response is almost equal but slightly in favor of the lower dose A bar graph depicting these data is shown in Figure 2

DISCUSSION

Despite the extensive publicity in recent years given poliomyelitis vaccine via Public Health and other channels in Minnesota, only slightly more than 40% of the girls in the group studied

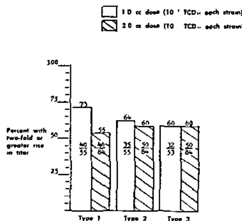


FIG 1 Comparison of percentages of two fold or greater response among two groups of pregnant women given two different doses of oral trivalent poliomyelitis vaccine

had received three or more injections of Salk vaccine Sixteen per cent had received only one or two injections Many others probably would not have obtained any Salk vaccine at all had they not become pregnant This reflects the *apathetic attitude prevalent in the population* as a whole to disease protection and illustrates the need for a poliomyelitis vaccine that is easily given and is capable of producing a durable protection by the administration of a single dose

TABLE 6. PERCENTAGE DISTRIBUTION OF POLIOVIRUS ANTIBODIES BY IMMUNOTYPE AND TITER AMONG 37 PREGNANT WOMEN WHO HAD NOT RECEIVED SALK VACCINE PRIOR TO VACCINATION WITH ORAL TRIVALENT VACCINE

TITER LEVEL	Antibodies, Type — BEFORE VACCINATION (NATURALLY OCCURRING) 1	2	3	AFTER VACCINATION 1	2	3
>1024	5 4	2 7	0 0	13 5	13 5	5 4
1024	0 0	8 1	0 0	8 1	13 5	2 7
256	24 3	27 0	18 9	43 2	43 2	27 0
64	29 7	24 3	13 5	27 0	13 5	29 7
16	8 1	10 8	16 2	5 4	8 1	24 3
4	5 4 } 40 5	0 0 } 37 8	10 8 } 67 5	2 7 } 8 1	8 1 } 16 2	8 1 } 35 1
<4	27 0	27 0	40 5	0 0	0 0	2 7

TABLE 7. PERCENTAGE DISTRIBUTION OF POLIOVIRUS ANTIBODIES BY IMMUNOTYPE AND TITER AMONG 186 PREGNANT WOMEN BEFORE AND 136 PREGNANT WOMEN AFTER TWO INJECTIONS OF COMMERCIAL SALK VACCINE [11]

TITER LEVEL	Antibodies, Type — NATURALLY OCCURRING 1	2	3	AFTER 2 SALK INJECTIONS 1	2	3
>1024	2 7	4 3	1 6	15 7	25 0	21 3
1024	4 3	11 3	7 0	22 4	22 8	24 3
256	13 5	15 0	15 6	13 4	21 3	19 8
64	12 9	15 6	17 2	18 7	16 2	8 8
16	21 0	9 1	11 3	11 9	12 5	11 8
4	10 7	13 4	7 5	6 7	1 5	3 7
<4	34 9	32 3	39 8	11 2	0 7	10 3

better than Type 1 and Type 3 All with unmeasurable titers to Type 1 and three of the four to Type 3 responded to vaccination with a fourfold or greater rise in titer. If two-fold increases in titer are considered there was a 100 per cent response to vaccination.

Among the 28 with two previous Salk injections, four had an unmeasurable antibody titer to a single immunotype. Two lacked antibody to Type 1 and two to Type 3 All of these responded to vaccination with a fourfold or greater increase in titer.

In the group of 57 who had received three or more Salk injections prior to oral vaccination there were 8 with an unmeasurable antibody titer to one or more immunotypes Three of these were to Type 1, three to Type 2, and six to Type 3 Of those with an unmeasurable titer to Type 1, all responded to the oral vaccine with a fourfold or greater increase in titer. Two of the three with an unmeasurable titer to Type 2, and five of the six with an unmeasurable titer to Type 3 also responded with a fourfold or greater rise in antibody titer.

There was one failure among those who had received one or more doses of Salk vaccine but

for each immunotype in each dose group were compared. When those with higher pre-vaccination antibody titers (1 64 or greater) were compared the superiority of the larger dose was not demonstrated

An analysis of the pre-vaccination antibody levels among those individuals studied by Cox and those reported here shows that the group that received the larger oral vaccination dose in this study included a much smaller proportion with unmeasurable titers than the group that received the smaller dose. These circumstances were reversed in the two groups compared by Cox. From these observations I was able to predict that a larger number of those studied by Cox had not received Salk vaccine prior to vaccination with trivalent oral vaccine This he has confirmed.

CONCLUSIONS

Trivalent poliomyelitis vaccine containing the three type strains of live attenuated virus has been fed to 223 young pregnant women without adverse effects or reactions seen or reported

The immunologic response seems equally good whether 1 0 cc or 2 0 cc of the vaccine is given although differences between the two groups given these doses is such that comparison of effect is difficult The larger dose appears to be slightly more antigenic if the pre-vaccination antibody titer is low

Not a single pregnant woman remained with an unmeasurable antibody titer to more than one immunotype after vaccination Among the entire group only two individuals remained with an unmeasurable titer to a single immunotype—one to Type 1 and another to Type 3.

Those pregnant women possessing only naturally-occurring antibodies (no Salk vaccine experience) responded to the trivalent oral vaccine in a superior manner when numbers of unmeasurable antibody titers before and after vaccination are compared to a similar group of individuals who received two injections of Salk vaccine

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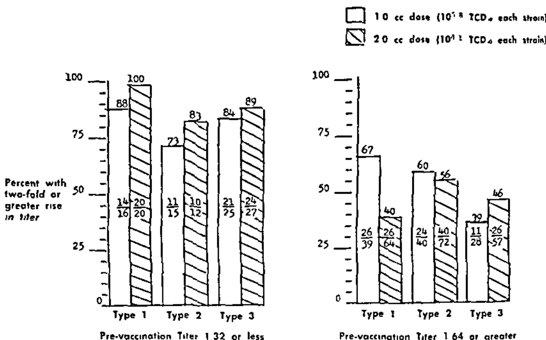


FIG 2 Comparison of numbers and percentages of two-fold or greater responses at two different pre-vaccination titer levels among two groups of pregnant women receiving two different doses of oral trivalent poliomyelitis vaccine

It is easier to produce significant rises in antibody levels by vaccination of individuals who have had a previous exposure to that antigen than it is to stimulate first antibody production in persons who have never had any previous exposure. That one feeding of this trivalent oral vaccine produces excellent results among those in need of vaccination is proved by the antibody response among those individuals in this study who had unmeasurable antibody titers prior to vaccination. Although more than one-half of those with unmeasurable antibody titers to each immunotype had no Salk injections prior to administration of the oral vaccine, 90 to 100% responded with a significant antibody titer rise This uniform response present even among those without previous Salk injections indicates that this oral trivalent vaccine is a potent antigen

Comparison of a group of non-Salk vaccinated pregnant women from this study who received oral vaccine with a similar group of women reported by da *Silva et al* [11] who received two doses of Salk vaccine during pregnancy shows fewer failures when conversion from unmeasurable to measurable titers are considered Granted that a third or booster injection of Salk vaccine would decrease the number of those failing to convert to a measurable titer. There is not sufficient time during pregnancy for a third injection if present recommended dosage schedules are adhered to

Because of this excellent immediate antibody response to a single dose of the trivalent oral poliomyelitis vaccine, the pregnant woman who does not report for her first prenatal visit until after her second missed period is now able to acquire protective antibodies before the third to fifth month of gestation when she is particularly susceptible to contracting the disease and in plenty of time for the last month of pregnancy when the mortality rate of acute poliomyelitis increases

Cox [8] found a considerably better antibody response for each immunotype among a group of 188 individuals fed $10^{8.1}$ TCD_{50} (2.0 cc) of each strain in a trivalent vaccine when compared to another group of 42 individuals fed one-half this dose. The results from the two groups in this study who received these same doses are not quite as clear cut Results similar to those of Cox were obtained, however, when those individuals with a low antibody titer (1:32 or less)

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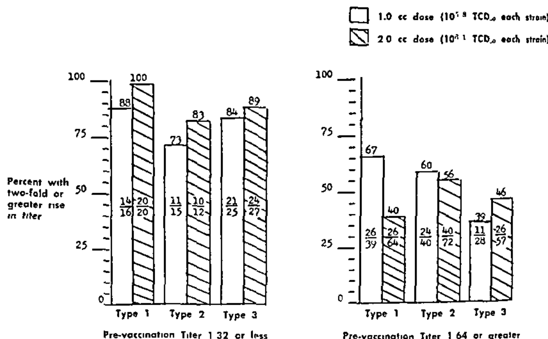


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Serological findings on the vaccinated group were found to be quite satisfactory

On the 25th of June Dr Voroshilova will present details of the results of our studies However, for the purpose of this discussion, I would like to mention certain of the highlights

So far only 62 pairs of sera, taken approximately one month apart, have been run The antibody titers after the administration of the trivalent mixture were actually higher than the titers following triple administration of the monovalent vaccine

Twelve triple negative children, when tested at 1 to 4 dilution acquired antibodies against all three types

Ten of the 12 acquired antibodies to Type 1 and Type 2, and all 12 developed antibodies against Type 3

Geometric mean antibody titers were 45 for Type 1, 208 for Type 2, and 97 for Type 3

We feel that the trivalent vaccine mixture has a definite place of promise because even in the case of the rare failures it was a relatively simple matter to revaccinate again, a much simpler procedure as compared to the other schemes

Our data are being confirmed by the presentations that are being made here

DR. COX I am not in a position to answer all these questions at the present time, because we have just finished the rough tabulation of the data concerning the 506 vaccinated individuals and are still in the process of further analysis

However, in regard to the 35 triple negatives I wish to state that all 35 developed antibody to Type 1 poliovirus One of the 35 subjects remained a double negative This person failed to develop antibodies to Types 2 and 3 In addition, 9 of the 35 persons failed to develop antibodies to the Type 2 component of the vaccine

We cannot see that the age of the individual makes much difference in regard to antibody response The ages of the triple negative individuals ranged from 12 to 51 years As mentioned previously 25 of the 35 triple negatives responded with antibodies to all three types of polioviruses.

Of the 9 persons who failed to respond to Type 2 alone, most are between 20 and 30 years old The single individual who remained a double negative and failed to respond to Types 2 and 3 is 31 years old

We will be able to give further information about these people later on because as I have stated, they are all employees of Lederle and we will be able to follow them rather carefully

DR RAMOS ALVAREZ I wish to add a few words on this subject Last year we reported at the VI International Congress of Tropical Medicine and Malaria in Lisbon the results of antibody studies in a group of 11 triple negative children to whom we had fed all 3 types of poliovirus simultaneously The strains used in our work were those developed by Dr Sabin Each type in the mixture was in a concentration of approximately $10^{5.5}$ to $10^{6.5}$ TCD_{50}

The tests on the pre and post feeding serum samples were carried out simultaneously The results we obtained are similar to the ones Dr Cox has reported We had 90 per cent response for Types 1 and 2 viruses and 63 per cent for Type 3 virus

I fully agree with Dr Sabin on the point that it is necessary to study virus excretion in the people fed the triple vaccine especially when they are in closed institutions However, I would like to add that in addition to this group of 11 children we have had the opportunity of studying another group of 45 children not living in closed institutions and the results we have had thus far are quite similar to the ones we had with the first group So I feel that the triple vaccine may work, provided each type of virus in the mixture is in a proper concentration

DISCUSSION

CHAIRMAN STUART-HARRIS: The papers presented by Dr Cox and Dr. Prem are open for discussion

DR. SABIN I think that Dr Cox and Dr Prem have done a most important job, because there is no question that from the point of view of practicability a decision will ultimately have to be faced by some, on the extent to which the benefits that might be derived from administering all three types at one time might outweigh the difficulties that would arise from immunization.

But, as a means of obtaining actual information, what happens when all three types are administered? The analysis, of necessity, needs to be limited to the triple negatives And to know what happens, one wants to know from the tables, not the ones that are no longer triple negatives, but rather how many of all the triple negatives used in the study that Dr. Cox and Dr Prem reported ended up with antibodies for all three types

The other point I wanted to make was that, in carrying out such a study for basic information on the behavior of the strains, it is extremely helpful to have actual data on the multiplication of the three viruses, as I showed in the tables the other day Because one must consider the fact that under conditions of family life, and sometimes even more so in institutions, in the presence of individuals who do not respond to one or more types, several of the viruses will be circulating and there will be the opportunity for picking up infection after interference is past

So, for basic information, one really needs to have the data on virus multiplication

The other point that I would like to make is that in the analysis of the significance of booster effects, it is very important to remember that when a person has antibody for two types and he is fed only the third type, and has multiplication of the type fed, a booster occurs in the other two types This is based on an analysis of data that we have from the study of 50 human volunteers *in the early period* of our work.

So that particularly in those who had lower naturally acquired titers to begin with, there was a boost in antibody, not only when Type 1 was fed for 2 and 3, but it appeared that each of the three types was capable of boosting the others

I would also hope that both Dr. Chumakov and Dr Smorodintsev, who have carried out similar tests with the three strains that I described, feeding all three types simultaneously, if they will not have an opportunity to present it at length in their own communications, may perhaps say a word about their results, which I have seen and I think have a bearing on this problem

DR CHUMAKOV *(through an interpreter)*: Application of a trivalent vaccine is very desirable from a number of standpoints in the first place, if there is too little time left before the usual poliomyelitis season begins, secondly, if it is necessary to immunize the greatest possible number of humans in the shortest time available, to interrupt the chain of human infection; and of course, thirdly, it is highly desirable to simplify the whole procedure of artificial immunization of humans.

Because of this, from the very beginning of our interest in poliomyelitis vaccine, concurrent studies have been in progress, not only on the triple administration of the monovalent vaccine, but on the single administration of the trivalent vaccine as well.

Insofar as we had on hand the Sabin attenuated strains, we felt safe in applying all three strains together as a single administration procedure Our hopes were fully confirmed

During the past five months, we have vaccinated over 400,000 children in the age group from two months to 15 years of age with the trivalent mixture

The mixture consisted of equal parts of each one of the poliovirus serotypes, containing .1 ml of each type, or approximately one million tissue culture infectious doses of each serotype

The majority of children received this mixture twice, about a month apart, roughly 65,000 children received the dose singly

There were positively no undesirable side effects during or after vaccination

Serological findings on the vaccinated group were found to be quite satisfactory

On the 25th of June Dr Voroshilova will present details of the results of our studies However, for the purpose of this discussion, I would like to mention certain of the highlights

So far only 62 pairs of sera, taken approximately one month apart, have been run The antibody titers after the administration of the trivalent mixture were actually higher than the titers following triple administration of the monovalent vaccine

Twelve triple negative children, when tested at 1 to 4 dilution, acquired antibodies against all three types

Ten of the 12 acquired antibodies to Type 1 and Type 2, and all 12 developed antibodies against Type 3

Geometric mean antibody titers were 45 for Type 1, 208 for Type 2, and 97 for Type 3

We feel that the trivalent vaccine mixture has a definite place of promise because even in the case of the rare failures it was a relatively simple matter to revaccinate again, a much simpler procedure as compared to the other schemes

Our data are being confirmed by the presentations that are being made here

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5. POLIOMYELITIS INFECTION RATE AMONG MEXICAN CHILDREN FED ATTENUATED POLIOVIRUS VACCINES *

a. Effect of Pre-existing Poliomyelitis Antibody
b. Interfering Role of Other Enteroviruses

MATILDA BENYESH-MELNICK, JOSEPH L. MELNICK, AND MANUEL RAMOS ALVAREZ

From the Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas, and the Virus Laboratory, Children's Hospital, Mexico City, Mexico

DR BENYESH-MELNICK *(presenting the paper)* In the spring of 1958 about 3,000 children were orally vaccinated in Mexico City by Dr. Manuel Ramos Alvarez with Sabin's attenuated poliovaccines[1] Families with more than one child, from a low socioeconomic group, were selected for the study The youngest child of each family was fed, first with Type 1 vaccine strain, followed by Type 3 and then Type 2 at three-week intervals, as recommended by Sabin A group of 91 families was selected for special study In 81 of the families the youngest child was fed the vaccine strains in the manner indicated above The remaining ten families in the group were not fed virus, and served as controls

Specimens for laboratory tests were collected from all the members of the 91 families as follows Rectal swabs—in 2 ml lactalbumin medium with 10X higher antibiotic concentration than ordinarily used[2]—were collected from the 81 vaccinated children, their family contacts, and the members of the 10 control families, before feeding and weekly for 9 weeks after the first feeding Serum samples were taken only from the 81 vaccinated children, before feeding, and at 3-week intervals for 9 weeks.

The antibody tests were carried out in the Virus Laboratory of the Children's Hospital, Mexico City. Serum titers were obtained by neutralization tests in tube cultures using the cytopathogenic endpoint. The rectal swabs were transported *on dry ice* to the Virus Laboratory, Baylor University College of Medicine, Houston, where the virus isolations and identifications were performed

PROCEDURES USED

Monkey kidney (MK) stationary tube cultures were used for virus isolations, titrations, and typings[2] They were prepared in M-H medium (0 5% lactalbumin hydrolysate in Hanks' salt solution) with 2% calf serum, and were maintained in M-E medium (0 5% lactalbumin hydrolysate in Earle's salt solution) with full 0 22% $NaHCO_3$ (pH 7 4 or above) and with 1% monkey serum and 0 15% bovine albumin

Isolations Each rectal swab suspension was adjusted to a volume of 3 ml. using M E medium as diluent. Then 0 1 ml of the specimen was inoculated into each of three MK tube cultures The tubes were kept at 36-37°C for a period of 2 weeks and readings for CPE (cytopathogenic effect) were performed on days 3, 5, 7, 9, 11, 13, and 15 after inoculation The positive specimens were harvested when showing 2+ to 3+ degeneration, and were stored at −20°C. Specimens harvested before the 7th day were titrated and typed For all the specimens harvested after the 7th day, an additional passage was made in an attempt to raise the titer before the virus was typed

Identification of the MK_1 or MK_2 isolates was performed by the CPE neutralization test in MK tubes, using 0.2 ml inoculum containing equal volumes of virus (100 TCD_{50}) and monkey antisera for polio Types 1, 2, and 3 (1:60 dilution) singly and as a mixture of the three sera The

* Aided by a grant from The National Foundation.

incubation period used for the virus + serum mixtures was one hour at 37°C. Each isolate was titrated in the same test to make sure that 100 TCD_{50} of virus had been used. The inoculated cultures were incubated at 36-37°C. for 9-11 days. Isolates neutralized by one of the sera and the mixture of the three were considered to belong to the respective type. Isolates which failed to be neutralized by the polio antisera were considered as non-polio enteroviruses, provided 100 TCD_{50} of the virus had been present in the test. All tests with more than 100 TCD_{50} of virus were repeated with the correct dose.

RESULTS

Tables 1 and 2 represent the results of virus isolations from the rectal swabs of the 81 vaccinated children.

Of 78 pre-feeding specimens, 68 were negative, 8 yielded non polio enteroviruses, and 2 yielded heterotypic wild polioviruses. If the excretion of a poliovirus began before the vaccine of that type was fed, it was considered a heterotypic, wild poliovirus.

Poliovirus excreted after vaccine feeding, and of the same type as the vaccine fed, was considered a homotypic poliovirus. Of the 596 post-feeding specimens tested, less than 50% (237) yielded a cytopathogenic agent in monkey kidney cultures. Of these, 40 specimens were homotypic Type-1, 25 homotypic Type-2, and 24 homotypic Type 3 polioviruses. The rate of infection was highest for Type-1. The majority of the Type-1 polioviruses were excreted in the first two weeks after feeding, although some were excreted as late as 5 weeks after feeding. The excretion of Type 3 appeared to be more prolonged than that of Type 1. No specimens were obtained later than 3 weeks after feeding Type-2, and therefore the data obtained may not give the true picture of duration of infection with this type.

TABLE 1. VIRUS ISOLATIONS FROM SPECIMENS OBTAINED FROM 81 CHILDREN FED SERIALLY WITH THE THREE TYPES OF POLIOVIRUS

Virus Isolated	Pre-Feeding	Post-Type 1			Post-Type 3			Post-Type 2			Post-Feeding Total
		7 da	14 da	21 da	7 da	14 da	21 da	7 da	14 da	21 da	
Homotypic* Polio											
Type 1	0	20	15	3	1	1	0	0	0	0	40
Type 2	0	0	0	0	0	0	0	12	8	5	25
Type 3	0	0	0	0	6	5	5	4	1	3	24
Heterotypic† Polio	2	1	0	2	2	3	1	2	2	0	13
Non-Polio	8	11	16	16	18	22	25	11	7	9	135
Total Pos	10	32	31	21	27	31	31	29	18	17	237
Total Neg	68	45	47	46	48	44	44	34	30	21	359
Total Spec Tested	78	77	78	67	75	75	75	63	48	38	596

* Homotypic indicates virus excreted after feeding was of same type as in vaccine fed.
† Heterotypic indicates excretion of a wild poliovirus of any type beginning before vaccine was fed.

TABLE 2 VIRUS ISOLATIONS FROM 81 CHILDREN ORALLY VACCINATED WITH TYPES 1, 2, AND 3

VIRUS ISOLATED	PRE-FEEDING	POST-TYPE 1	POST-TYPE 3	POST-TYPE 2	TOTAL POST-FEEDING
Homotypic Polio*					
Type 1	0	38	2	0	40
Type 2	0	0	0	25	25
Type 3	0	0	16	8	24
Heterotypic Polio†	2	3	6	4	13
Non-Polio	8	43	65	27	135
Total Positive	10	84	89	64	237
Total Negative	68	138	136	85	359
Total Tested	78	222	225	149	596

* Homotypic indicates virus excreted after feeding was of same type as in vaccine fed.
† Heterotypic indicates excretion of a wild poliovirus of any type beginning before vaccine was fed

Of the 237 positive post-feeding specimens, 13 were wild heterotypic polioviruses Non-polio enteroviruses constituted 57% (135) of the positive specimens, suggesting the possibility of their interfering effect upon infection with the viruses fed This aspect will be discussed later in this paper. Of the 596 specimens tested, 359 did not yield any cytopathogenic agent However, some of these specimens possibly contained agents which were present in concentrations too low to be detected in monkey kidney cultures, or which were not cytopathogenic for monkey kidney cells and therefore were missed

The patterns of virus excretion for individual children are presented in Tables 3, 4, and 5

Table 3 contains a sampling of the results on children who did not excrete virus in their pre-feeding specimens One can observe the variety of patterns of virus excretion after vaccine feeding While some of the children were infected with two of the viruses fed, others did not become infected with any of the vaccine viruses Of these, some became infected with naturally occurring non polio enteroviruses, and the failure of the vaccine to produce infection could be explained by the interfering effect of the non-polio viruses occupying the intestinal tract. In other children, the failure of infection with the vaccine viruses was due to prior infection with polioviruses, as revealed by the high antibody level for the appropriate type before feeding

For example, child No 15 in Table 3 was a 3 month old baby, with high antibody level for Type-3 and no antibodies for Type 1 and Type 2, in the pre-feeding samples. The child failed to become infected with any of the three viruses administered, the reason being, for Type-3, the high type-specific antibody level before feeding and for Types 1 and 2, the interfering effect of the non-polio virus infection present at the time of feeding and after

Table 4 presents the patterns of virus isolation from the children who excreted non polio viruses in their pre-feeding specimens

The pattern does not differ much from that of children with negative pre-feeding specimens However, there was more interference between the non-polio viruses and the vaccine virus, for fewer homotypic infections took place in these children. The ones who became infected were infected with only one of the vaccine strains, with a much shorter duration of virus excretion

TABLE 3 PATTERNS OF VIRUS ISOLATIONS FROM CHILDREN WHO DID NOT EXCRETE VIRUS IN THEIR PRE-FEEDING SPECIMENS

CHILD No	PRE-FEEDING	TYPE OF VIRUS ISOLATED FROM SPECIMENS								
		POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
372	0	P1	P1	0	P3	P3	P3	0	0	—
51	0	0	0	—	P3	0	0	P2	0	—
376	0	P1	P1	0	0	0	NP	—	—	P2
244	0	0	0	0	0	0	0	0	P2	P2
132	0	0	0	0	0	0	0	0	0	0
15	0	NP	NP	0	0	0	0	NP	NP	NP
93	0	NP	NP	NP	NP	0	NP	P2	—	—
107	0	0	0	0	P3	P3	B5	—	P3	0
268	0	P1	P1	0	0	NP	NP	P2	P2	0
4	0	P1	P1	P1	P1	NP	P3	P3	—	—

P1 = Polio Type 1, P2 = Polio Type 2, P3 = Polio Type 3
NP = Non Polio Enterovirus
B5 = Coxsackie B5

0 = Negative
— = No specimen

TABLE 4 PATTERNS OF VIRUS ISOLATIONS FROM CHILDREN WHO EXCRETED NON POLIO VIRUSES IN THEIR PRE-FEEDING SPECIMENS

CHILD No	PRE-FEEDING	TYPE OF VIRUS ISOLATED FROM SPECIMENS								
		POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
86	NP	0	0	B5	NP	NP	NP	0	0	0
157	NP	0	0	0	0	NP	NP	0	0	P2
277	NP	NP	NP	—	—	—	0	0	0	—
281	NP	0	0	0	0	0	NP	NP	E-15	—
285	NP	P1	P1	NP	0	0	0	0	0	0
295	NP	NP	NP	0	NP	—	NP	0	0	0
358	NP	P1	NP	0	0	NP	0	NP	NP	NP

P1 = Polio Type 1, P2 = *Polio Type 2, P3 = Polio Type 3*
NP = Non Polio Enterovirus
B5 = Coxsackie B5
E 15 = ECHO virus type 15

0 = Negative
— = No specimen

Table 5 includes the results of isolations from children who excreted heterotypic polioviruses

As mentioned earlier, we considered as heterotypic any poliovirus whose excretion began before the corresponding vaccine type was fed. There were 13 such isolates in the post-feeding specimens and two in the pre-feeding specimens, all obtained from 5 children The typing of all the heterotypic polioviruses was repeated and confirmed The details of this table have been discussed in our earlier paper at this Conference With the exception of the two Type-1 strains excreted by child No. 102 on the 7th and 14th days after Type-1 feeding, all the strains in the table were considered as heterotypic wild polioviruses

Table 6 contains data on the types of virus infection in the vaccinated children, based on virus isolation alone. Of 81 vaccinated children, only 40 excreted homotypic polioviruses Of these, 13 excreted homotypic poliovirus alone; 26 excreted homotypic polio plus non-polio virus, and one excreted homotypic and heterotypic polioviruses, plus a non-polio virus Not a single child in the study was infected with all the three vaccine strains administered, as can be seen from the bottom row in the table Sixteen children were infected with two of the vaccine viruses, and the remaining 24 exhibited single type infection only

The relationship of age to the type of virus infection in the vaccinated children is dealt with in Table 7

Of 21 children under 6 months tested, 14 excreted homotypic polioviruses Of these, 12, or

TABLE 5 VIRUS ISOLATIONS FROM CHILDREN WHO EXCRETED HETEROTYPIC POLIOVIRUSES

Child No		Type of Virus Isolated From Specimens									
		Pre-Feeding	Post-Type 1			Post-Type 3			Post-Type 2		
			7 DA	14 DA	21 DA	7 DA	14 DA	21 DA	7 DA	14 DA	21 DA
65	type	0	0	0	—	0	—	P2	P2	P2	NP
	char *							d+	d+	d+	
99	type	NP	NP	NP	P3	P3	P3	0	B5	NP	—
	char				d+	d+	d+				
102	type	0	[P1]	[P1]	P3	P3	P3	0	0	NP	—
	char		[d]	[d]	d+	d+	d+				
224	type	P3	P3	NP	NP	NP	P3	0	0	—	0
	char	d+	d+				d+				
380	type	P1	0	0	NP	NP	0	0	P1	P1	--
	char	d							d	d	

* Character of virus [illegible] ch grow well at low bicarbonate concentrations under agar Such strains are
d+ [illegible]
d [illegible] under agar

P1=Polio Type 1; P2=Polio Type 2, P3=Polio Type 3
NP=Non Polio enterovirus
B5=Coxsackie B5
0=Negative
—=No specimen
[ ]=homotypic poliovirus (i e., virus excreted after feeding was of same type as in the vaccine fed)

TABLE 6 VIRUS TYPES ISOLATED FROM 81 ORALLY VACCINATED CHILDREN

Virus Types in Positive Children					Negative
Homotypic* Polio	Homotypic Polio + Non-Polio	Homotypic Polio + Heterotypic† Polio + Non-Polio	Heterotypic Polio + Non-Polio	Non-Polio	
13	26	1	4	24	13

Subtotal (Homotypic Polio, Homotypic Polio + Non-Polio, Homotypic + Heterotypic + Non-Polio): 40

P1, P2, P3	P1, P2	P1, P3	P2, P3	P1	P2	P3
0	8	7	1	8	10	6

* Homotypic indicates virus excreted after feeding was of same type as in vaccine fed
† Heterotypic indicates excretion of a wild poliovirus of any type beginning before vaccine was fed

TABLE 7 RELATIONSHIP OF AGE TO TYPE OF VIRUS INFECTION IN 81 ORALLY VACCINATED CHILDREN

Age	Number of Children	Number of Children With Homotypic Polio Infection	Per Cent With		
			Single Polio Infection	Double Polio Infection	Triple Polio Infection
0-6 months	21	14	22	78	0
7 months-12 years	60	26	81	19	0

78%, were doubly infected, that is to say, excreted two of the three types fed. Of 60 children above six months of age, 26 excreted polioviruses. Of these, 81% had single polio infection and only 19% had double polio infections.

The higher susceptibility of children under six months of age to the vaccine viruses is even more apparent from the data illustrated in Table 8.

In this study each child was exposed to poliovirus three times (one for each vaccine strain) and theoretically had the opportunity for triple infection. Taking this fact into consideration, the number of children tested in each age group was multiplied by 3 to obtain the total number of exposures to poliovirus. The total number of homotypic polio infections (based on virus isolation only) in this case was obtained from the number of children with single infections plus the number of children with double infections times 2 (i.e., a child excreting 2 types of vaccine virus was counted twice). As mentioned above, triple infection was not observed in any member of the group studied. In the table the per cent of homotypic polio infections based on number of children tested (left-hand side of Table 8) is compared with the per cent based on the total number of exposures to poliovirus (right-hand side of the table). From the data obtained on the

TABLE 8 HOMOTYPIC POLIO INFECTIONS * IN DIFFERENT AGE GROUPS OF VACCINATED CHILDREN, DETERMINED ON THE BASIS OF NUMBER OF CHILDREN TESTED, AND ON THE BASIS OF NUMBER OF THEIR EXPOSURES TO POLIOVIRUSES

Age Group	Number of Children Tested	Children With Homotypic Polio Infections		Number of Exposures to Polioviruses†	Homotypic Polio Infections			
		Number	Per Cent of Total Tested		Number: Single	Number: Double	Total Number‡	Per Cent of Total Exposures
months								
0–6	21	14	67	63	3	11	25	40
7–12	12	6	50	36	4	2	8	22
13–18	15	7	47	45	7	0	7	16
19–35	15	6	40	45	5	1	7	16
years								
3–5	13	6	46	39	4	2	8	21
6–12	5	1	20	15	1	0	1	7
Total	81	40	50	243	24	16	56	23

* Infections as determined by isolation only.
† Obtained by multiplying number of children x 3 (3 exposures per child)
‡ Double infections are included twice in the totals (i.e., infection with 2 types is counted as 2 infections)
No triple infections were found

basis of number of children tested, there was no significant difference in the per cent of children excreting homotypic poliovirus for the various age groups up to the age of five years, the per cent slightly decreasing with age. However, there was a marked drop in the incidence of infections in the 6-12 year-old group. On the other hand, when the results were analyzed on the basis of number of exposures, there was a sharp decrease in the incidence of infection after the age of 6 months (from 40% homotypic infections for the children under 6 months to 22% for the next older group). Comparing the total figures, on the basis of number of children tested, 50% of the children excreted poliovirus, however, on the basis of exposures, only 23% of the chances for infection were actually realized. An analysis based on exposures reveals a more accurate picture of the degree of infection taking place in the vaccinated children.

The pattern of immunity in this group of children immediately before feeding was typical for a population of this geographic area and socio-economic level. Figure 1 illustrates the polio Type-1 antibody patterns prior to vaccine feeding. One can see from the curves that the loss of ma

FIG. 1. Polio Type 1 antibody patterns prior to vaccine feeding. Mexico City, 1958

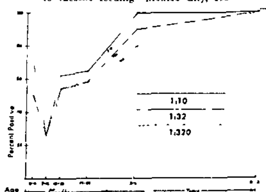


TABLE 9 RELATIONSHIP OF AGE TO PREVALENCE AND DEVELOPMENT OF POLIO-1 ANTIBODIES AFTER INGESTION OF TYPE 1 POLIOVIRUS VACCINE

AGE	PER CENT OF TOTAL TESTED IN EACH AGE GROUP			TOTAL NUMBER OF CHILDREN TESTED
	WITH HIGH HOMOTYPIC* ANTIBODY BEFORE VACCINE FEEDING	WITH ANTIBODY† RISE AFTER VACCINE FEEDING	WITH NO HOMOTYPIC‡ ANTIBODIES BEFORE OR AFTER VACCINE FEEDING	
Months				
0– 6	50	40	10	20
7–12	28	36	36	11
13–18	55	30	15	13
19–35	64	29	7	14
Years				
3– 5	90	0	10	10
6–12	100	0	0	3

* 41 children had the antibody pattern indicated
† 20 " " " " " "
‡ 10 " " " " " "

ternal antibody by the 7th month after birth is soon followed by natural infection and acquisition of immunity By the age of one year, about 50% *of the children have already had natural infection* with Type-1 By the age of 3-5 years, 90% of the children had been infected and had high levels of immunity The pattern for Type-2 is very similar to that of Type-1, with immunity present at high levels by the age of 18 months For Type 3 the susceptible period is prolonged until the age of 3 years However, it should be emphasized that 81 children constitute a small sample of the population in the area, even though it included all in our study group

The relationship of age to prevalence and development of polio antibodies after vaccine ad*ministration is shown in the following* three tables Table 9 is concerned with the results for Type-1

Seventy-one children were tested They were divided into three groups: children with high homotypic antibody before vaccine feeding, those with antibody rise after vaccine feeding, and those with no homotypic antibodies before or after vaccine feeding Fifty per cent of the 20 children under 6 months of age in the test group had high homotypic antibodies before vaccine feeding (presumably maternal), 40% did not have prior antibodies and responded with antibody rise after feeding; and 10% did not show antibodies either before or after feeding After *the age* of one year, the per cent of children with high antibody level increased progressively reaching 100% for the 6-12-year old group Consequently, the percentage of children responding with antibody *rise to the vaccine decreased with* age. Of special interest in this study are the children represented in the third column of the table, i e, children who were free of antibodies in their pre-feeding specimens, and yet did *not* exhibit an antibody response to the vaccine The high percentage of non-polio enteroviruses circulating in this population probably accounts for the latter findings, on the basis of the interfering effect of the non-polio viruses upon the vaccine viruses administered Tables 10 and 11 represent the data for Type 2 and Type-3 antibody status The percentage of children who failed to respond to the vaccines was even greater for these two types Since the vaccines were administered in the order Type 1, Type-3, Type-2, an additional interfering effect of previously fed vaccine virus, still multiplying in the intestinal tract at the time of a second vaccine feeding, could very well be responsible for this finding.

TABLE 10 RELATIONSHIP OF AGE TO PREVALENCE AND DEVELOPMENT OF POLIO-2 ANTIBODIES AFTER INGESTION OF TYPE 2 POLIOVIRUS VACCINE

AGE	PER CENT OF TOTAL TESTED IN EACH AGE GROUP			TOTAL NUMBER OF CHILDREN TESTED
	WITH HIGH HOMOTYPIC* ANTIBODY BEFORE VACCINE FEEDING	WITH ANTIBODY† RISE AFTER VACCINE FEEDING	WITH NO HOMOTYPIC‡ ANTIBODIES BEFORE OR AFTER VACCINE FEEDING	
Months				
0- 6	53	20	27	15
7-12	66	17	17	6
13-18	18	27	55	11
19-35	84	8	8	13
Years				
3- 5	74	13	13	8
6-12	100	0	0	3

* 34 children had the antibody pattern indicated
† 9 " " " " " "
‡ 13 " " " " " "

TABLE 11 RELATIONSHIP OF AGE TO PREVALENCE AND DEVELOPMENT OF POLIO-3 ANTIBODIES AFTER INGESTION OF TYPE 3 POLIOVIRUS VACCINE

AGE	PER CENT OF TOTAL TESTED IN EACH AGE GROUP			TOTAL NUMBER OF CHILDREN TESTED
	WITH HIGH HOMOTYPIC* ANTIBODY BEFORE VACCINE FEEDING	WITH ANTIBODY† RISE AFTER VACCINE FEEDING	WITH NO HOMOTYPIC‡ ANTIBODIES BEFORE OR AFTER VACCINE FEEDING	
Months				
0- 6	44	17	39	18
7-12	13	25	62	8
13-18	25	33	42	12
19-35	50	14	36	14
Years				
3- 5	100	0	0	9
6-12	100	0	0	3

* 31 children had the antibody pattern indicated
† 11 " " " " " "
‡ 22 " " " " " "

The relationship between homotypic virus isolation and antibody status in the vaccinees is shown in Table 12

If we consider the data for Type-1, of the 40 children who had high level antibodies before vaccination, *only 5 excreted virus* (all children under 6 months of age), and 35 failed to excrete virus Similar findings were obtained for Types 2 and 3

Of the children showing antibody rise to the vaccine virus, 28 excreted and 9 failed to excrete virus in their stools The latter finding might be due to our failure to isolate virus from the rectal swab specimens There was a high correlation between the failure to produce antibodies after vaccination and the failure to excrete homotypic virus in the children studied. This raises again the question of the interfering effect of non-polio enteroviruses (see Table 13).

The children tested for all three types were divided into two groups: those who did not excrete non-polio viruses before vaccine feeding, and those who did Of the 28 children in the first group, 22 showed homotypic antibody rise Poliovirus was isolated from 18 of the 22 Six children in this group did not produce antibodies but did not excrete homotypic virus, either In the second group—the children who excreted non-polio viruses before vaccine feeding—the situation was reversed Most of these children (33 of 47 tested) did not produce antibodies and did not excrete homotypic virus (30 of the 33), as a result of the interfering effect of the non-polio viruses occupying their intestinal tracts However, *this did not take place in all children* infected with non-polio viruses. Thus, of the remaining 14 children in the group, who in spite of infection with other enteroviruses did produce polio antibodies, 10 excreted homotypic virus also This suggests that *only certain* enteroviruses may act as interfering agents for poliovirus, or that timing and dosage play a role as has been observed in quantitative experiments on poliovirus interference in tissue culture[3]

Thus far we have discussed the factors responsible for actual infection with oral vaccines in children of different age groups in a population of low socioeconomic level with early natural

TABLE 12 RELATIONSHIP BETWEEN VIRUS ISOLATION AND ANTIBODY STATUS IN ORALLY VACCINATED CHILDREN

Antibody Status	Number of Children Who Excreted Homotypic Polioviruses				Number of Children Who Failed To Excrete Homotypic Polioviruses				Total
	P-1	P-2	P-3	Total	P-1	P-2	P-3	Total	
High Homotypic Antibody Level Before Vaccine Feeding	5	3	2	10	35	31	28	94	104
Homotypic Antibody Rise After Vaccine Feeding	16	6	6	28	4	2	3	9	37
No Homotypic Antibodies Before or After Vaccine Feeding	0	1	2	3	10	12	20	42	45
Total Tested				41				145	186

TABLE 13 INFLUENCE OF INFECTION WITH OTHER ENTEROVIRUSES BEFORE VACCINE FEEDING ON HOMOTYPIC ANTIBODY PRODUCTION AGAINST VACCINE VIRUS

ANTIBODY STATUS	NO OF CHILDREN WHO DID NOT EXCRETE OTHER VIRUSES BEFORE VACCINE FEEDING			NO OF CHILDREN WHO EXCRETED OTHER VIRUSES BEFORE VACCINE FEEDING		
	TOTAL	EXCRETED POLIOVIRUS	DID NOT EXCRETE POLIOVIRUS	TOTAL	EXCRETED POLIOVIRUS	DID NOT EXCRETE POLIOVIRUS
Homotypic Antibody Rise After Feeding	22	18	4	14	10	4
No Homotypic Antibodies Before Or After Feeding	6	0	6	33	3	30
Total	*28*	*18*	*10*	*47*	*13*	*34*

polio infection and an abundance of other enteroviruses Another important factor which remains to be discussed is the degree of spread of the vaccine viruses to the immediate contacts of the vaccinees

In our study, rectal swabs were collected from the family contacts in the same manner and at the same intervals as from the 81 vaccinees No blood samples were taken and no antibody studies were carried out on the contacts, and therefore all the results are based on virus isolation and identification alone Table 14 represents the results on 227 family contacts in different age groups as compared to those of the 81 children vaccinated

While 50% of the vaccinees excreted homotypic poliovirus after feeding, only 7% of the family members became infected as a consequence of contact. *In the contacts, too, the* highest per cent of virus excretion was observed in children under the age of five years The incidence of non-polio enterovirus infection was the same for contacts as for the vaccinees Some of the family contacts were infected with wild heterotypic polioviruses, mainly the children under the age of 3 *The right hand side of the* table represents data on 38 members of 10 control families in which no children were fed. No polioviruses were isolated from these subjects, 26% of them carried other, non-polio entero viruses. Another group of 100 young children in Mexico City was sampled just prior to the initiation of this study They yielded 23 enteroviruses, none being poliovirus

In Table 15, the data on isolations from vac cinees and contacts are analyzed in the same fashion as in Table 8 discussed above, namely on the basis of individuals tested, and on the basis of the number of their exposures to vaccine virus

We would like to stress again the relatively low number of actual infections taking place in vaccinees—only 23% of the potential exposures to the vaccine viruses were realized, as compared to the 50% when the data are calculated on the basis of the individuals tested The spread of vaccine virus to the family contacts appears to be minimal indeed in this group—being only 3% realization of the theoretically possible exposures The reasons for this are at least threefold. (1) not all vaccinated children became carriers, (2) the contacts had a high degree of natural im munity, and (3) the contacts were also heavily infected with interfering non-polio enteroviruses

SUMMARY AND CONCLUSIONS

This paper serves as an example of the problems that are encountered when oral poliovirus

TABLE 14 RELATIONSHIP OF AGE TO VIRUS EXCRETION IN 81 VACCINATED CHILDREN, IN THEIR 227 FAMILY CONTACTS, AND IN 38 MEMBERS OF 10 CONTROL FAMILIES IN WHICH NO MEMBER WAS VACCINATED

AGE GROUP (YRS)	VACCINATED CHILDREN				FAMILY CONTACTS				MEMBERS OF CONTROL FAMILIES	
	NUMBER TESTED	PER CENT IN EACH AGE GROUP EXCRETING VIRUS INDICATED			NUMBER TESTED	PER CENT IN EACH AGE GROUP EXCRETING VIRUS INDICATED			NUMBER TESTED	PER CENT IN EACH AGE GROUP EXCRETING VIRUS
		HOMOTYPIC POLIO*	HETEROTYPIC POLIO†	NON-POLIO		HOMOTYPIC POLIO*	HETEROTYPIC POLIO†	NON-POLIO		NON-POLIO
<3	63	52	6	32	22	18	5	45	11	45
3–5	13	46	0	23	50	16	0	50	5	60
6–12	5	20	0	20	77	1	1	20	11	18
>12	0	0	0	0	78	4	1	12	11	0
Total	81	50	5	29	227	7	1	26	38	26

* Homotypic indicates virus excreted after feeding was of same type as in vaccine fed
† Heterotypic indicates excretion of a wild poliovirus of any type beginning before vaccine was fed

TABLE 13. INFLUENCE OF INFECTION WITH OTHER ENTEROVIRUSES BEFORE VACCINE FEEDING ON HOMOTYPIC ANTIBODY PRODUCTION AGAINST VACCINE VIRUS

ANTIBODY STATUS	No of Children Who Did Not Excrete Other Viruses Before Vaccine Feeding			No of Children Who Excreted Other Viruses Before Vaccine Feeding		
	Total	Excreted Poliovirus	Did Not Excrete Poliovirus	Total	Excreted Poliovirus	Did Not Excrete Poliovirus
Homotypic Antibody Rise After Feeding	22	18	4	14	10	4
No Homotypic Antibodies Before Or After Feeding	6	0	6	33	3	30
Total	28	18	10	47	13	34

polio infection and an abundance of other enteroviruses Another important factor which remains to be discussed is the degree of spread of the vaccine viruses to the immediate contacts of the vaccinees

In our study, rectal swabs were collected from the family contacts in the same manner and at the same intervals as from the 81 vaccinees No blood samples were taken and no antibody studies were carried out on the contacts and therefore all the results are based on virus isolation and identification alone Table 14 represents the results on 227 family contacts in different age groups as compared to those of the 81 children vaccinated

While 50% of the vaccinees excreted homotypic poliovirus after feeding, only 7% of the family members became infected as a consequence of contact *In the contacts too, the* highest per cent of virus excretion was observed in children under the age of five years The incidence of non polio enterovirus infection was the same for contacts as for the vaccinees. Some of the family contacts were infected with wild heterotypic polioviruses, mainly the children under the age of 3. *The right hand side* of the table represents data on 38 members of 10 control families in which no children were fed. No polioviruses were isolated from these subjects: 26% of them carried other, non-polio enteroviruses Another group of 100 young children in Mexico City was sampled just prior to the initiation of this study They yielded 23 enteroviruses, none being poliovirus

In Table 15, the data on isolations from vaccinees and contacts are analyzed in the same fashion as in Table 8 discussed above, namely on the basis of individuals tested, and on the basis of the number of their exposures to vaccine virus

We would like to stress again the relatively low number of actual infections taking place in vaccinees—only 23% of the potential exposures to the vaccine viruses were realized, as compared to the 50% when the data are calculated on the basis of the individuals tested The spread of vaccine virus to the family contacts appears to be *minimal indeed in this group—being only 3%* realization of the theoretically possible exposures The reasons for this are at least threefold. (1) not all vaccinated children became carriers, (2) the contacts had a high degree of natural immunity; and (3) the contacts were also heavily infected with interfering non-polio enteroviruses

SUMMARY AND CONCLUSIONS

This paper serves as an example of the problems that are encountered when oral poliovirus

vaccine is used in a subtropical area One cannot assume that mere feeding of the vaccine will be sufficient to produce infection and immunity in the children

This study points up two factors, one of which has been well established by previous studies in the epidemiology of poliomyelitis, and has been discussed by earlier speakers at this Conference This is the fact that children possessing antibodies as a result of natural infection are immune to reinfection In contrast, children with maternal antibodies proved to be as fully susceptible as children without antibodies.

The second factor is now being brought into focus by studies being reported at this Conference, namely that current infection with nonpolio enteroviruses interferes with the establishment of poliovirus infection even in children free of polio antibodies

It should be emphasized that information on this point has been established and can be further defined only by detailed laboratory analysis of sufficient material collected during field trials Field observations alone, of vaccinated children, cannot supply the necessary data

This study raises also the question as to the interpretation of some of the field trials already carried out, many of which were done in areas where the level of natural immunity is high, and where enterovirus infections are very frequent

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3 Ledinko, N, and Melnick, J L. Interference between poliomyelitis viruses in tissue culture J Exp M, *100:* 247-267, 1954

TABLE 15. HOMOTYPIC POLIO INFECTIONS* IN DIFFERENT AGE GROUPS OF VACCINATED CHILDREN AND THEIR CONTACTS, DETERMINED ON THE BASIS OF NUMBER OF INDIVIDUALS TESTED, AND ON THE BASIS OF NUMBER OF THEIR EXPOSURES TO POLIOVIRUSES

Age Group (yrs)	Vaccinated Children				Contacts			
	Number of Children Infected / Tot Tested	Per Cent	Number of Homotypic Infections† / Tot Exposures‡	Per Cent	Number of Individuals Infected / Tot Tested	Per Cent	Number of Homotypic Infections / Tot Exposures	Per Cent
<3	33/63	52	47/189	25	4/22	18	5/66	8
3 5	6/13	46	8/39	21	8/50	16	8/150	5
6 12	1/5	20	1/15	7	1/77	1	1/231	<1
>12	0	0	0	0	3/78	4	3/234	1
Total	40/81	50	56/243	23	16/227	7	17/681	3

* Infections as determined by isolation only
† Double infections are included twice in the totals (i e, infection with 2 types is counted as 2 infections) No triple infections were found
‡ Obtained by multiplying number of children x 3 (3 exposures per child)

TABLE 1 INCIDENCE OF POLIOMYELITIS IN SINGAPORE FROM 1946 UP TO PERIOD OF THE EPIDEMIC

YEAR	JAN, FEB, MARCH	APRIL, MAY, JUNE	JULY, AUG, SEPT	OCT, NOV, DEC
1946	187	1	—	—
1947	—	—	—	—
1948	—	119	33	20
1949	21	14	23	20
1950	4	3	8	75
1951	30	20	16	28
1952	21	20	11	4
1953	11	11	17	8
1954	36	29	13	7
1955	15	7	2	1
1956	6 (1)	12 (2)	3 (1)	51 (1)
1957	27 (1)	13 (1 & 3)	14 (3)	10 (3)
1958	2 (1 & 3)	3 (1)		

The figures in brackets indicate the serological type of poliomyelitis isolated during the period

In view of this situation, the Minister of Health in the Singapore Government decided to offer, on a purely voluntary basis, immunization with the attenuated poliomyelitis virus vaccine to children between three months and 10 years

Although the epidemic was due to the Type 1 virus and all reported cases were shown to be infected with this serological type, it was decided to use a vaccine of the Type 2 attenuated virus for the following reasons

(a) Any large-scale use of attenuated virus vaccine at this stage should be such that it would be possible to arrive at a conclusion at the end of the trial as to the safety of the vaccine not only for the vaccinees but also their *contacts* who became secondarily infected

All cases of poliomyelitis that had occurred in Singapore since 1956 had been examined to establish the serological type of the virus responsible Table 1 shows that cases due to Type 2 virus had not been found since June 1956, and as every case was examined by laboratory procedures, any due to Type 2 virus would have been spotted immediately in this Type 1 epidemic Had Type 2 cases occurred they would have been assumed to have resulted from the introduction of the vaccine strain into the population, especially had there been any significant *number of such* cases,

(b) Feeding of attenuated strains of all three types simultaneously in chimpanzees (Sabin 1956) resulted in a complete suppression of multiplication of Type 3 virus Interference of one serological type with the establishment of a second serological type in the *alimentary tract* was the possibility that resulted in the suggested administration of one serological type of attenuated vaccine at one time It was hoped that children in whom the attenuated Type 2 strain was established would show this interference phenomenon if exposed to the Type 1 strain,

6. LARGE-SCALE USE OF SABIN TYPE 2 ATTENUATED POLIOVIRUS VACCINE IN SINGAPORE DURING A TYPE 1 POLIOMYELITIS EPIDEMIC

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Dr Hale *(presenting the paper)* In the latter half of 1958 Singapore experienced an epidemic outbreak of poliomyelitis due to the Type 1 virus The incidence of paralytic poliomyelitis, by week of onset, and the age distribution of paralytic cases are shown in Figures 1 and 2, respectively

The second report of the World Health Organization Expert Committee on Poliomyelitis (1958) suggested that in the face of an impending epidemic or where poliomyelitis of the infantile type is endemic, especially where signs are indicative of an imminent shift to the epidemic form of the disease, a large-scale trial of attenuated vaccine might be attempted

Experience of poliomyelitis in Singapore since 1946 is shown in Table 1

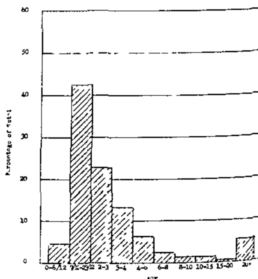


Fig 2 Age distribution paralytic cases

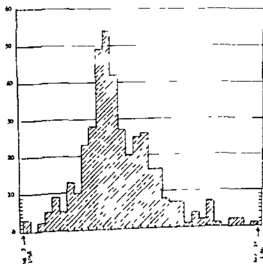


Fig. 1 Incidence of paralytic poliomyelitis by week of onset

Paul (1958) drew attention to the fact that this endemic state of poliomyelitis was associated with a high infantile mortality rate and if the infantile mortality rate fell below 60 80 per 1,000 live births, a rise in the number of cases of poliomyelitis could be expected The infantile mortality rates for Singapore since 1945 are shown in Table 2

This fall in the infantile mortality rate could presage a shift to the direction of increased activity of poliomyelitis and the possible appearance of cases in older children A serological survey of the population possessing poliomyelitis antibodies (Table 3) was conducted in 1956 The proportion of children with poliomyelitis antibodies was relatively low, confirming the possibility of epidemic conditions arising

(c) Persons who have experienced poliomyelitis infection often show some heterologous protection against the other types. Second paralytic attacks of poliomyelitis, although theoretically possible, are extremely rare. Experimentally Sabin (1956) and Koprowski (1955) showed that the dose of an attenuated virus necessary to *establish an alimentary infection was greater in* the person with heterotypic antibody than those devoid of all antibody. Vaccination would ensure that all vaccinees had Type 2 antibodies and this heterotypic protection might be invoked.

(d) The large-scale use of the vaccine would result in dissemination of large quantities of attenuated virus throughout the community and *this virus could interfere with the natural transmission* of the prevalent epidemic strain

TABLE 3 SEROLOGICAL SURVEY (SINGAPORE)—APRIL 1956—POLIOMYELITIS

Age-Group	Sera Tested	Positives			Percentage Positive		
		Type 1 Antibody	Type 2 Antibody	Type 3 Antibody	Type 1 Antibody	Type 2 Antibody	Type 3 Antibody
3/12–6/12	6	1	0	3	16 7	——	50 0
7/12–23/12	10	1	1	4	10 0	10 0	40 0
2–3 years	8	5	4	4	62 5	50 0	50 0
3–4 years	12	8	7	11	75 0	58 3	91 7
4–6 years	26	16	16	17	61 5	61 5	68 9
6–8 years	38	28	25	32	73 7	66 6	85 2
8–10 years	16	8	12	9	50 0	75 0	56 3
10–20 years	23	17	16	13	73 9	69 6	56 5
20+ years	138	74	81	59	53 6	58 7	42 7

TABLE 4 ANTIBODY SURVEY OF POPULATION JUST BEFORE COMMENCING VACCINATION

Age-Group	No Tested	Antibodies			Percentage Positive		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
3/12–6/12	25	11	9	7	44 0	36 0	28 0
7/12–23/12	71	23	9	18	32 4	12 7	25 4
2–3 years	60	34	15	33	56 7	25 0	55 0
3–4 years	56	42	33	33	75 0	58 9	58 9
4–6 years	71	53	53	54	74 6	74 6	76 1
6–8 years	70	52	61	62	74 3	87 1	88 6
8–10 years	83	68	79	77	81 9	95 2	92 8

The percentage of children susceptible to Type 1 and also Type 2 infection is illustrated in Table 5

TABLE 2 INFANT MORTALITY RATE PER 1,000 LIVE BIRTHS IN SINGAPORE SINCE 1945

	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957
Chinese	199 52	82 03	79 43	71 02	64 51	75 13	66 69	62 27	58 22	46 80	40 36	33 97	32 81
Malays	289 78	140 23	143 25	155 27	119 99	141 72	136 75	120 01	124 38	106 71	104 61	95 46	86 40
Indians	200 66	98 31	76 45	76 79	80 89	67 56	70 66	66 19	62 94	60 76	44 15	34 27	39 64
Europeans } Eurasians	224 81	53 98											
Europeans			57 69	43 10	19 61	29 82	23 19	31 70	25 79	21 37	17 42	16 41	17 54
Eurasians			77 99	16 24	35 33	52 96	36 65	47 35	73 85	35 93	33 24	24 86	27 78
Others	182 48	143 68	113 51	61 90	86 02	83 33	92 04	78 28	60 12	63 06	57 72	29 95	37 61
Total	215 71	89 69	87 33	80 79	72 04	82 23	75 15	69 97	67 04	56 10	49 67	42 66	41 11

(c) Persons who have experienced poliomyelitis infection often show some heterologous protection against the other types. Second paralytic attacks of poliomyelitis, although theoretically possible, are extremely rare. Experimentally Sabin (1956) and Koprowski (1955) showed that the dose of an attenuated virus necessary to establish an alimentary infection was greater in the person with heterotypic antibody than those devoid of all antibody. Vaccination would ensure that all vaccinees had Type 2 antibodies and this heterotypic protection might be invoked;

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		Type 1 Antibody	Type 2 Antibody	Type 3 Antibody	Type 1 Antibody	Type 2 Antibody	Type 3 Antibody
3/12–6/12	6	1	0	3	16 7	—	50 0
7/12–23/12	10	1	1	4	10 0	10 0	40 0
2–3 years	8	5	4	4	62 5	50 0	50 0
3–4 years	12	8	7	11	75 0	58 3	91 7
4–6 years	26	16	16	17	61 5	61 5	68 9
6–8 years	38	28	25	32	73 7	66 6	85 2
8–10 years	16	8	12	9	50 0	75 0	56 3
10–20 years	23	17	16	13	73 9	69 6	56 5
20+ years	138	74	81	59	53 6	58 7	42 7

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7/12–23/12	71	23	9	18	32 4	12 7	25 4
2–3 years	60	34	15	33	56 7	25 0	55 0
3–4 years	56	42	33	33	75 0	58 9	58 9
4–6 years	71	53	53	54	74 6	74 6	76 1
6–8 years	70	52	61	62	74 3	87 1	88 6
8–10 years	83	68	79	77	81 9	95 2	92 8

The percentage of children susceptible to Type 1 and also Type 2 infection is illustrated in Table 5

TABLE 2 INFANT MORTALITY RATE PER 1,000 LIVE BIRTHS IN SINGAPORE SINCE 1945

	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957
Chinese	199.52	82.03	79.43	71.02	64.51	75.13	66.69	62.27	58.22	46.80	40.36	33.97	32.81
Malays	289.78	140.23	143.25	155.27	119.99	111.72	136.75	120.01	124.38	106.71	104.61	95.46	86.10
Indians	200.60	98.31	76.45	76.79	80.89	67.56	70.66	66.19	62.94	60.76	44.15	34.27	39.64
Europeans }	224.81	53.98	57.69	43.10	19.61	29.82	23.19	31.70	25.79	21.37	17.42	16.41	17.54
Eurasians }			77.99	46.24	35.33	52.96	36.65	47.35	73.85	35.93	33.24	24.86	27.78
Others	182.48	143.68	113.51	61.90	86.02	83.33	92.04	78.28	60.12	63.06	57.72	29.95	37.61
Total	215.71	89.69	87.33	80.79	72.04	82.23	75.15	69.97	67.04	56.10	49.67	12.66	41.11

TABLE 6 EXCRETION OF ENTERIC VIRUSES BY HEALTHY CHILDREN IMMEDIATELY PRIOR TO VACCINATION CAMPAIGN

Age-Group	No Tested	Virus Isolations				Percentage of Non-Polio Enteric Virus Excreters	Percentage of Polio Type 1 Excreters
		Coxsackie Type A	Coxsackie Type B or A9	ECHO	Polio Type 1		
0–3/12	19	2	0	3	0	26 3	0 0
3/12–6/12	42	2	3	2	1	16 1	2 4
7/12–23/12	71	5*	3	2	3*	14 1	4 2
2–3 years	57	6	3	7	2	28 1	3 5
3–4 years	31	1	1	3	1	16 1	3 2
4–6 years	28	1	0	1	0	7 1	0 0
Total	248	17*	10	18	7*	18 1	2 8

* One child was excreting Polio Type 1 virus and a Coxsackie Type A

and the results are shown in Table 6 (This survey also indicated the dissemination of Type 1 strain in the population)

The vaccine which was obtained through the kind offices of Dr A Sabin was given in 0 1 ml quantities of a 1/10 dilution of the tissue culture fluid in a teaspoonful of B P Syrup Simplex Children attending the centers set up by the Singapore Government Health Department had their temperatures taken before receiving the vaccine Those with raised temperatures were excluded in an attempt to weed out those who might be incubating poliomyelitis

Excretion of Type 2 virus by vaccinees

The excretion of Type 2 virus by vaccinees was followed in a number of cases Table 7 shows this rate of excretion, and as was expected the percentage of excreters in each group corresponded very closely to the percentage in each group shown to be susceptible by serological tests

TABLE 7 EXCRETION RATE OF TYPE 2 VIRUS BY VACCINEES

Age-Group	No Tested	No Excreting Type 2 Virus	Percentage
3/12–6/12	36	21	58 3
7/12–3/12	95	53	55 8
2–3 years	70	38	54 3
3–4 years	63	30	47 6
4–6 years	106	25	23 6
6–8 years	83	12	14 5
8–10 years	82	16	19 5

Degree of antibody response to vaccination by Type 2 susceptibles

Children bled prior to vaccination, whose sera contained no detectable Type 2 antibody were selected for further study Feces collected at intervals of time were tested for the presence of Type 2 virus and at the end of three to four weeks a second serum was examined for the

TABLE 5. ANALYSIS OF POLIO TYPE 1 SUSCEPTIBLES

Age-Group	No Tested	No Susceptible Type 1	Percentage	Type 1 Susceptible No Type 2 or 3 Antibody	Percentage of Number Tested	Type 1 Susceptible No Type 2 Antibody	Percentage of No Tested
3/12–6/12	25	14	56 0	8	32 0	14	56 0
7/12–23/12	71	48	67 6	40	56 3	47	66 2
2–3 years	60	26	43 3	10	16 7	23	38 7
3–4 years	56	14	25 0	2	3 6	6	10 7
4–6 years	71	18	25 4	2	2 8	6	8 5
6–8 years	70	18	25 7	2	2 8	2	2 8
8–10 years	83	15	18 1	0	0 0	1	1 2

Immediately prior to the commencement of the vaccination campaign, which was started after the epidemic had reached its eleventh week, a serological survey was conducted to ascertain what proportion of the children who were to receive the vaccine would be susceptible to Type 2 strain, and how many susceptible to Type 1 virus were also susceptible to Type 2 virus It was only in this latter group that an interference phenomenon could be expected to play a part in protection A number of children were bled just before administration of the vaccine and the type of poliomyelitis antibodies present in their sera ascertained Table 4 shows the percentage of children with antibodies against each type of virus.

It was apparent that there was an increase in the percentage of children with poliomyelitis antibodies since the 1956 survey was conducted The increase in immunity to Type 1 virus was an indication of its circulation in the community as the survey was commenced only after the epidemic had reached its twelfth week Unfortunately many of the population have serious objections—some religious, some superstitious—to the bleeding of their children As a result of this, our survey could only be completed over a period of a few weeks and during this period popular demand was such that the vaccination campaign had to proceed This was indicated in the survey figures as a rise in the number of children with Type 2 antibodies due to contact with vaccinees In 40 cases we found the apparently anomalous result of children with Type 2 antibodies present in their sera when the vaccine was given, and yet, Type 2 virus was found in their feces tested 10 days later A second specimen of serum taken three to four weeks after vaccination in all these cases showed four- to thirty two fold rise in the titer of neutralizing antibodies present. We concluded that these were children who were already infected at the time of vaccination, presumably from contact with a vaccinee

Excretion of enteric viruses in the child population

Sabin (1958a) reported several patterns of interference with multiplication of attenuated poliovirus in some children who were carriers of non poliomyelitis enteric viruses prior to vaccination In view of this it was decided to ascertain the degree of enteric non-poliomyelitis virus excretion in our child population to determine, if possible the effect of this on the vaccination program Feces from children from birth to four years old were collected and examined for the presence of enteric viruses

TABLE 9 MONKEY PATHOGENICITY TESTS—POST-VACCINE TYPE 2 ISOLATES

AGE	DAYS AFTER TAKING VACCINE VIRUS ISOLATED	DOSE X Log10 TCD_{50}	ROUTE OF	RESULT
5 mths	8	5 0	IS	0/2
6 mths	8	5 5	IS	0/2
7 mths	11	4 5 3 5 5 2	IS IS IC	1/2 0/2 0/2
8 mths	12	5 0	IS	0/2
10 mths	23	5 33	IS	0/2
17 mths	8	5 33	IS	0/2
2 yrs	5	5 33 4 33 6 03	IS IS IC	1/2 1/2 0/2
2 yrs 5 mths	11	5 33	IS	0/2
2 yrs 10 mths	5	5 0 4 0 5 7	IS IS IC	1/2 0/2 0/2
4 yrs	5	4 23	IS	0/2
4 yrs	8	4 5 3 5 5 2	IS IS IC	1/2 0/2 0/2
4 yrs 7 mths	8	5 66 4 66 6 36	IS IS IC	1/2 1/2 0/2
6 yrs 11 mths	11	5 0	IS	0/2
7 yrs	8	5 5	IS	0/2
7 yrs 8 mths	8	5 5	IS	0/2
8 yrs 2 mths	8	5 5	IS	0/2
8 yrs 2 mths	8	5 0 4 0 5 7	IS IS IC	1/2 0/2 0/2
8 yrs 5 mths	8	5 5	IS	0/2

* Numerator=Number of monkeys paralyzed Denominator=Number of monkeys inoculated

TABLE 8. ANTIBODY RESPONSE TO VACCINATION

Age-Group	No of Children Without Type 2 Antibody	No With Type 2 Antibody 3–4 Weeks After Oral Vaccine	Percentage Response	No of Children	Titer*
3/12–6/12	10	7	70.0	2	50
				2	112
				2	250
				1	564
7/12–23/12	44	38	86.4	1	2
				1	10
				5	22
				3	50
				4	112
				2	250
				8	564
				5	1250
				9	1250+
2–3 years	32	31	96.9	4	2
				4	10
				5	22
				4	50
				5	112
				1	250
				3	564
				2	1250
				3	1250+
3–4 years	18	17	94.4	1	2
				1	10
				4	22
				3	112
				3	250
				4	564
				1	1250
4–6 years	14	13	92.9	1	2
				1	22
				2	50
				5	112
				2	564
				1	1250
				1	1250+
6–8 years	5	5	100.0	1	50
				2	112
				1	250
				1	1250+
8–10 years	2	2	100.0	1	22
				1	564

* Titer is expressed as the reciprocal of the highest dilution of the serum neutralizing 100 TCD_{50} of the Type 2 virus (0.1 ml of serum used in control).

TABLE 9 MONKEY PATHOGENICITY TESTS—POST-VACCINE TYPE 2 ISOLATES

AGE	DAYS AFTER TAKING VACCINE VIRUS ISOLATED	DOSE X LOG10 TCD_{50}	ROUTE OF	RESULT*
5 mths	8	5.0	IS	0/2
6 mths	8	5.5	IS	0/2
7 mths	11	4.5 3.5 5.2	IS IS IC	1/2 0/2 0/2
8 mths	12	5.0	IS	0/2
10 mths	23	5.33	IS	0/2
17 mths	8	5.33	IS	0/2
2 yrs	5	5.33 4.33 6.03	IS IS IC	1/2 1/2 0/2
2 yrs 5 mths	11	5.33	IS	0/2
2 yrs 10 mths	5	5.0 4.0 5.7	IS IS IC	1/2 0/2 0/2
4 yrs	5	4.23	IS	0/2
4 yrs	8	4.5 3.5 5.2	IS IS IC	1/2 0/2 0/2
4 yrs 7 mths	8	5.66 4.66 6.36	IS IS IC	1/2 1/2 0/2
6 yrs 11 mths	11	5.0	IS	0/2
7 yrs	8	5.5	IS	0/2
7 yrs 8 mths	8	5.5	IS	0/2
8 yrs 2 mths	8	5.5	IS	0/2
8 yrs 2 mths	8	5.0 4.0 5.7	IS IS IC	1/2 0/2 0/2
8 yrs 5 mths	8	5.5	IS	0/2

* Numerator=Number of monkeys paralyzed Denominator=Number of monkeys inoculated

presence and titer of Type 2 antibody The results of this study are shown in Table 8

Spread of the vaccine strain among the contacts of vaccinees

From several Child Welfare Clinics in widely different areas of the island, feces were collected from children who had not received vaccine but were reporting on account of minor illness or for medical check-ups Of 633 children tested, 14 were found to be excreting the Type 2 virus and 23 the Type 1 strain, which was a clear indication that the vaccine strain spread through the population as readily as the epidemic Type 1 strain This spread of the vaccine strain among contacts was also confirmed, as stated earlier, by the finding of attenuated Type 2 virus excretion in 40 children whose sera prior to vaccination revealed Type 2 antibody but sera titrated 3-4 weeks later showed a four- to thirty-two-fold increase in antibody titer

Pathogenicity of Type 2 strains excreted

Virus obtained from vaccinees of various ages and at different periods of time following vaccination were tested for pathogenicity in monkeys and the results are shown in Table 9 Pathogenicity tests were also carried out on Type 2 viruses isolated from contacts who had not received vaccine (Table 10)

TABLE 10 MONKEY PATHOGENICITY TESTS

(Virus isolated from children who have become infected from others)

AGE	DOSE X LOG 10 TCD_{50}	ROUTE OF INNOCULATION	RESULT*
3 mths	5 23	IS	0/2
8 mths	4 23	IS	1/2
	3 23	IS	0/2
	4 93	IC	0/2
8 mths	4 5	IS	0/2
14 mths	4 5	IS	0/2
1 yr 7 mths	5 33	IS	0/2
1 yr 8 mths	4 5	IS	0/2
3 yrs	5 0	IS	0/2
3 yrs 1 mth	5 33	IS	0/2
8 yrs	6 33	IS	1/2
	5 33	IS	1/2
		IC	0/2

* Numerator=Number of monkeys paralyzed Denominator=Number of monkeys inoculated

DISCUSSION

A virus of attenuated virulence to be used as a vaccine must give rise to no illness or upset in the person inoculated or infected with the material The attenuated Type 2 poliovirus used in this instance was administered to 198,965 *children between the ages of three months and* 10 years without causing any cases of paralytic poliomyelitis within the group This finding demonstrates the safety of this vaccine for the vaccinee In other trials (Sabin, 1956, 1957a and b, 1958, Verlinde et al, 1958) no untoward effects resulted in vaccinees from the use of attenuated virus We were only able to follow carefully a limited number of children after vaccination but in all instances of children excreting the virus, we observed no ill effects, they remained perfectly fit, afebrile and continued a normal life. Young children in Singapore suffer from constipation, diarrhea, and run temperatures from colds, etc, as they do elsewhere, but it must be realized that these incidents present one of the minor difficulties of a mass campaign as mothers usually ascribe these symptoms to the vaccine if they follow its administration

One of the great problems in the use of attenuated poliovirus vaccine is that of virus excretion by the susceptible person, which often results in infection of contacts and the danger is that a mutant virulent strain will arise Our results showed quite clearly that the vaccine strain spread rapidly through the susceptible members of the community Many small-scale experiments have been made in which attenuated virus was fed to volunteers and the excreted virus tested for pathogenicity, and several workers (Sabin, 1957a; Dane et al., 1957, Dick & Dane, 1957; Clarke et al., 1958) have reported the isolation of strains of increased pathogenicity.

Our results also showed some strains with slight increase in pathogenicity but there was no evidence whatsoever that the alimentary tract was selective for this type of variant. Tests on strains isolated from contacts where the virus must have had at least one human passage gave the same picture of occasional increase in pathogenicity, but these passaged strains showed no greater increase in pathogenicity than those obtained direct from vaccinees. The age of the child, the question of previous poliomyelitis experience and the time lapse after vaccination that the virus was isolated, did not appear to be the factor determining excretion of more pathogenic variants. It cannot be overemphasized that this increase in virulence is a laboratory measurement using the extremely sensitive monkey lumbar spinal cord neurons. The real question at issue is not that of pathogenicity to monkey lumbar cord neurons but the capacity to cause disease in man. Strains of greater virulence than that exhibited by excreted strains of this vaccine have been isolated from perfectly healthy children during inter-epidemic periods. Smorodintsev et al. (1958) reported studies on triple negative children fed Type 1 vaccine strain supplied by Dr. Sabin. While they observed a slight increase in neurotropism of the excreted virus it was no greater after an estimated four to five natural human passages than it was in the stools of the children receiving the original culture. Using Type 2 strain used in the campaign we have described, these workers found that in the course of five experimental consecutive passages in triple negative children, there was a partial increase in both cerebral and spinal neurotropism in the stools of the fourth passage, but this was no longer found at the fifth passage.

In this trial 198,965 children were vaccinated and a large percentage excreted virus which spread rapidly through the community, an ideal situation to estimate the theoretical danger discussed above. One case of paralytic poliomyelitis due to Type 2 virus in a four year old child occurred in the twentieth week of the epidemic, nine weeks after the commencement of vaccinations. It was an isolated case and if it had resulted from infection by an excreted vaccine strain many more cases would have been expected. Although Type 2 virus had not been isolated from paralytic cases in Singapore since June 1956, such cases were occurring in Malaya even at the time of the commencement of the Type 1 epidemic in Singapore. At this time several strains of Type 2 poliovirus were isolated from healthy children in Johore Bahru (the neighboring town in Malaya separated from Singapore by the causeway). Pathogenicity tests of these strains are shown in Table 11 and there seems little doubt that "wild" virulent strains were circulating in this area. It is therefore highly probable that the one case reported in Singapore resulted from infection with one of these "wild" strains.

TABLE 11. MONKEY PATHOGENICITY TESTS OF TYPE 2 VIRUS FOUND IN HEALTHY CHILDREN IN JOHORE BAHRU AT COMMENCEMENT OF VACCINATION CAMPAIGN IN SINGAPORE

AGE	DOSE X LOG 10 TCD_{50}	ROUTE OF INOCULATION	RESULT*
3 mths	5.33	IS	2/2
	4.33	IS	2/2
	6.03	IC	1/2
1 yr	5.33	IS	0/2
2 yrs 2 mths	6.0	IS	1/2
	5.0	IS	1/2
	6.7	IC	0/2
3 yrs 1 mth	5.0	IS	2/2
	4.0	IS	2/2
	5.7	IC	2/2

* Numerator=Number of monkeys paralyzed. Denominator=Number of monkeys inoculated.

Following the safety of the vaccine the most important characteristic is the degree of immunity that it will confer on an individual. In this trial, for reasons already stated, it was decided to use a Type 2 vaccine that could only give a heterologous protection and possibly an interference phenomenon against the current Type 1 epidemic strain. The degree of homologous protection could only be assessed by the conventional laboratory method of estimating the antibody response in Type 2-susceptible children fed the vaccine strain. The antibody titers attained in these children showed the expected

presence and titer of Type 2 antibody The results of this study are shown in Table 8

Spread of the vaccine strain among the contacts of vaccinees

From several Child Welfare Clinics in widely different areas of the island, feces were collected from children who had not received vaccine but were reporting on account of minor illness or for medical check ups Of 633 children tested, 14 were found to be excreting the Type 2 virus and 23 the Type 1 strain, which was a clear indication that the vaccine strain spread through the population as readily as the epidemic Type 1 strain This spread of the vaccine strain among contacts was also confirmed, as stated earlier, by the finding of attenuated Type 2 virus excretion in 40 children whose sera prior to vaccination revealed Type 2 antibody but sera titrated 3-4 weeks later showed a four to thirty-two-fold increase in antibody titer

Pathogenicity of Type 2 strains excreted

Virus obtained from vaccinees of various ages and at different periods of time following vaccination were tested for pathogenicity in monkeys and the results are shown in Table 9 Pathogenicity tests were also carried out on Type 2 viruses isolated from contacts who had not received vaccine (Table 10)

TABLE 10. MONKEY PATHOGENICITY TESTS

(Virus isolated from children who have become infected from others)

AGE	DOSE X LOG 10 TCD_{50}	ROUTE OF INNOCULATION	RESULT*
3 mths	5 23	IS	0/2
8 mths	4 23 3 23 4 93	IS IS IC	1/2 0/2 0/2
8 mths	4 5	IS	0/2
14 mths	4 5	IS	0/2
1 yr 7 mths	5 33	IS	0/2
1 yr 8 mths	4 5	IS	0/2
3 yrs	5 0	IS	0/2
3 yrs 1 mth	5 33	IS	0/2
8 yrs	6 33 5 33	IS IS IC	1/2 1/2 0/2

* Numerator=Number of monkeys paralyzed Denominator=Number of monkeys inoculated

DISCUSSION

A virus of attenuated virulence to be used as a vaccine must give rise to no illness or upset in the person inoculated or infected with the material The attenuated Type 2 poliovirus used in this instance was administered to 198,965 children between the ages of three months and 10 years without causing any cases of paralytic poliomyelitis within the group This finding demonstrates the safety of this vaccine for the vaccinee. In other trials (Sabin, 1956, 1957a and b, 1958; Verlinde et al, 1958) no untoward effects resulted in vaccinees from the use of attenuated virus We were only able to follow carefully a limited number of children after vaccination but in all instances of children excreting the virus, we observed no ill effects, they remained perfectly fit, afebrile and continued a normal life. Young children in Singapore suffer from constipation, diarrhea, and run temperatures from colds, etc., as they do elsewhere, but it must be realized that these incidents present one of the minor difficulties of a mass campaign as mothers usually ascribe these symptoms to the vaccine if they follow its administration

One of the great problems in the use of attenuated poliovirus vaccine is that of virus excretion by the susceptible person, which often results in infection of contacts and the danger is that a mutant virulent strain will arise Our results showed quite clearly that the vaccine strain spread rapidly through the susceptible members of the community. Many small-scale experiments have been made in which attenuated virus was fed to volunteers and the excreted virus tested for pathogenicity, and several workers (Sabin, 1957a, Dane et al., 1957; Dick & Dane, 1957; Clarke et al, 1958) have reported the isolation of strains of increased pathogenicity

established itself and both Types 1 and 2 virus were found in the feces This superimposed infection of Type 2 was also seen in one other case and the interesting feature of this case is that serum taken 49 days after vaccination showed a 125 fold rise of Type 1 neutralizing antibodies but no detectable Type 2 antibody in a 1 in 5 dilution of the serum although the Type 2 strain had infected the alimentary tract

SUMMARY

An epidemic outbreak of poliomyelitis due to a Type 1 strain occurred in Singapore during the latter months of 1958 and first two months of 1959 A very definite trend to cases in older children was noticed, previous outbreaks had always been in locally domiciled children under about two to three years old This is discussed and the suggestion is made that Singapore is changing from an area where poliomyelitis is endemic and cases of the infantile type occur, to one in which epidemics might be expected and older children would be involved

A decision was made at government level to offer an attenuated Type 2 vaccine supplied by Dr Sabin for voluntary vaccination The experimental organization and results of this campaign are discussed Of 198,965 children vaccinated, only six developed paralysis due to Type 1 virus During the same period of time, that is one week after the start of the vaccination campaign to the close of the epidemic, there were 179 paralytic Type 1 cases in approximately 300 000 non vaccinated children of the same age-group

No untoward effects followed the vaccination and no case of Type 2 paralysis occurred in the vaccinees Antibody response to the vaccine was highly satisfactory although there were a small proportion of children in the younger age groups who failed to give detectable antibodies following vaccination Possible reasons for this are discussed in the text

Some of the virus strains excreted by vaccinees showed slight increases in virulence to monkeys tested by intraspinal inoculation This was not a progressive phenomenon and strains isolated from contacts, where it could be assumed that at least one human passage had taken place were no more virulent that those obtained directly from vaccinees

Only one case of Type 2 paralytic poliomyelitis occurred in a non-vaccinated child and, as it was shown, that wild virulent Type 2 strains were circulating in a neighboring community, it was thought that this case could well have resulted from infection with such a wild strain

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scatter and titers had no observable relationship to the age of the child or their previous experience of poliomyelitis infection In the younger age-groups the percentage responding was lower than in the older age-groups and the possible reasons for this are somewhat difficult to elucidate It might be that such children were actually infected, but virus excretion was transitory and antibody response unmeasurable by our technique Susceptible cells in these children may, however, have undergone such change that they were no longer capable of reinfection with that particular serological type of virus Alternatively it may represent a true failure of the vaccine strain to cause infection Sabin (1958) showed that ECHO 9 and a virus isolated in ERK cells interfered with multiplication of the Type 3 vaccine strain The proportion of non-poliomyelitis enteric viruses excreted was found to be higher in the younger than in the older age-groups. However, in cases we were able to follow closely, children excreting Coxsackie type A, Coxsackie Types A9 or B or ECHO viruses, which unfortunately we were not in a position to classify further became infected by Type 2 strain when given vaccine and their antibody responses were of the same order found in other children In no case did we find any interference with the establishment of Type 2 virus, in fact although it was easy to isolate the non poliovirus prior to vaccination, four days later, when the Type 2 strain was established, however carefully we tried we failed to isolate the strain found before vaccination Nevertheless it could well be that, as we used only monkey kidney cells, we failed to detect viruses that interfered with the establishment of Type 2 vaccine strain. Refeeding at a later date of those children who failed to respond should decide whether despite a failure to detect antibody they are nevertheless immune or that there was a true failure of the vaccine strain to infect

The protection afforded by this Type 2 vaccine strain against Type 1 epidemic strain is difficult to interpret as vaccination proceeded over a period of weeks and a proportion of the theoretically non-vaccinated population actually became vaccinated as the result of infection contracted from vaccinees. During the course of the epidemic six cases of paralytic poliomyelitis due to Type 1 developed among 198,965 vaccinated children (this figure excludes the cases incubating Type 1 infection when given the vaccine) During the same period from the first week after commencement of the vaccination campaign there were 179 paralytic Type 1 cases in approximately 300,000 non-vaccinated children of the same age-groups as the vaccinees. The age distribution of children was approximately the same in vaccinated and non-vaccinated groups These figures are probably biased against showing protective effects of the vaccine because a proportion of the 300,000 non-vaccinated children were in fact secondarily vaccinated As there were no grounds to suppose that these secondarily vaccinated children would not have the same protection shown by the primarily vaccinated, the difference between vaccinated and non-vaccinated groups was probably much greater On the other hand, it can quite rightly be stated that any child whose parents intended that it should be vaccinated but who developed poliomyelitis before this, would be automatically included in the non-vaccinated group This would result in a slight rise in the paralytic case ratio in the non-vaccinated group Although the majority of the vaccinations were completed in seven weeks as long as the public demanded it centers remained open, so that it was not until 11 January 1959 that the last vaccination was completed By this time the epidemic was very much on the wane which was no doubt the reason that there were no further requests for vaccination Two weeks after the last vaccination was performed there were 13 paralytic cases all in non-vaccinated children These cases must have become infected after vaccinations had ceased and it does represent, therefore a relatively uncomplicated comparison between vaccinated and non-vaccinated children

One significant finding was that none of the six cases of paralytic poliomyelitis among vaccinees occurred within the period 10-20 days following vaccination This is the period when any interference effect of Type 2 virus would be expected to be maximal Type 1 virus appeared *to exert very little interference on Type 2 virus* One child was paralyzed when presented for vaccination although this was not spotted until just after the vaccine was given At the time of vaccination Type 1 virus was being excreted but within four days the vaccine strain had

difficult, and I would like to have his opinion as to how he assesses the effect of the Type 2 feeding on the Type 1 cases, especially in the light of all the variations that one might expect in terms of racial distribution age specific rates, the point he raised of the recruitment of individuals into the non-vaccinated case group because they had not quite gotten the vaccine—all of these factors

Could he attempt some sort of a measure of the effectiveness of the procedure, or would he prefer to say that he cannot measure it at all?

Dr Hale I do not think that I can go into greater analysis. I agree, on looking back, that there are many things that one would like to have done, such as giving a placebo But I think with the condition existing, that would have been quite impossible because of the emotional atmosphere

Dr Sabin: Also, placebo would not have helped very much because the vaccinated and the non-vaccinated children also contracted some Type 2 infection, as Dr Hale has shown So the placebo approach does not lend itself to analysis here

But there is one point I would like to make which is contained in the orginal report, namely that in the Type 1 cases that did occur in the vaccinated children, there is an interval of about 10 to 20 days, an interval of time in which the greatest multiplication of Type 2 might have been expected and when no Type 1 cases occurred, this is in line with the suggestion that that is a period during which perhaps, the greatest effect of interference might have occurred

There are two possible effects, one by interference, immediately after extensive multiplication of the virus, and the other by this partially protective effect that several people have now demonstrated, both experimentally and epidemiologically

Dr Payne I would like to ask Dr Hale whether there was any sign that the epidemic was particularly heavy in any specific area of the island and whether there was any sign that the vaccine distribution on the island differed at all from that?

Dr Hale: There was no sign that the epidemic was greater in any particular area To a great extent, it was that the number of cases were largely dependent upon the population figures of that area, and vaccination was spread pretty widely throughout the island

Dr. Bodian I am afraid that Dr Hale did not answer my question I think that he is better aware of the circumstances of this study than anybody else in this room, and therefore I would like to get his frank opinion concerning the force, if any, of the protective effect

It seems to me that he is the one who has to make some sort of assessment, as quantitative as possible, of the force of an assumed protective effect, because obviously there have been variables unmeasured and unmeasurable.

Dr Hale Well, my opinion is that there was a protective effect in the vaccinated group

Dr Gear: I would like to ask Professor Hale if the virus isolated from the one Type 2 case is still extant, and secondly, if that case had any brothers or sisters, from whom specimens were taken for examination?

Dr Hale I believe the strain is still extant

Chairman Stuart Harris I would like to make a comment here in relation to Dr Bodian's remarks

Dr Hale presented his results a month ago, at a meeting of some of the people in England interested in live poliovirus vaccines and we did discuss the question of the evaluation of the protection The manner in which the feeding was done was over a period of some weeks after the epidemic had begun, and the statisticians present at the meeting felt that it was almost impossible to take any cases occurring in the non-vaccinated groups, in comparing those with the numbers in the vaccinated groups, until after the period of time when all these administrations of vaccine had been completed

That is why I think Dr Hale commented that after that period there were 13 further cases in non-vaccinated children, at which time the two groups should have been capable of being compared

DISCUSSION

CHAIRMAN STUART-HARRIS: The papers presented by Dr. Benyesh-Melnick and Dr. Hale are open for discussion

DR PLOTKIN: Dr Hale, what was the ethnic distribution of the cases of polio in Singapore during the epidemic?

DR HALE. The detailed distribution is given in a full report in the *British Medical Journal* However, I think I can give you those figures in just a minute

CHAIRMAN STUART-HARRIS Can you give him one of the reprints?

DR HALE Yes

DR. PLOTKIN Were they predominantly in one group?

DR HALE: No The European population was higher but remember that is a small population

DR PLOTKIN Were the vaccinations concentrated in any ethnic group, or were they distributed in all?

DR HALE: They were distributed, and the proportion was about the same in each group, not just the European group

DR BARR: As a health officer, I would like to state that this is an excellent study Why did you not feed the children Type 1 vaccine in order to control the obvious Type I outbreak?

DR HALE: Then I might have been in difficulty sorting out I would not have known future types, and what cases were due to my vaccine strain or the wild strain

DR BARR: I asked you as a health officer, not as a scientist.

DR. HALE: I am not a health officer, and therefore can only answer as a scientist

CHAIRMAN STUART-HARRIS Dr Sabin

DR. SABIN. I would like to answer Dr. Barr by saying that, at the time this study was made in October of last year, we had no data on the safety of this Type 1 vaccine during non epidemic periods, and I personally refused to have the first large-scale trial of the Type 1 vaccine made in an epidemic area where we would have no end of trouble trying to explain the cases that subsequently occurred

DR. FOX: I was one of those who earlier raised the question of the possible intermixture of wild strains with homotypic strains, or vaccine strains in the Mexican trials

I would simply like to say that I think the data presented by Dr Benyesh-Melnick suggest rather strongly that the great proportion of the strains which she considered to be homotypic probably are homotypic and vaccine-derived

However, I would like to say that Drs Melnick and Benyesh-Melnick have some equivalent material from our own study, and I would be rather interested in the results that they may one of these days obtain

DR MELNICK But you never did send us your material

DR FOX You will certainly receive it

DR BENYESH-MELNICK: I am glad that our presentation convinced Dr. Fox at last that the virus excreted by the vaccinated children usually had its origin in the vaccine that was fed

DR. BODIAN: I would like, first of all, to congratulate Dr. Hale on a very interesting approach and a bold one I think the central issue here concerns the effect of the Type 2 feeding on the course of the epidemic.

Dr. Hale was kind enough to let me see the full report, which appears in the *British Medical Journal*. It is clear that the anlysis of frequency of occurrence of cases in the immunized and in the so-called unvaccinated groups is extremely

difficult, and I would like to have his opinion as to how he assesses the effect of the Type 2 feeding on the Type 1 cases, especially in the light of all the variations that one might expect in terms of racial distribution, age specific rates, the point he raised of the recruitment of individuals into the non vaccinated case group because they had not quite gotten the vaccine—all of these factors

Could he attempt some sort of a measure of the effectiveness of the procedure, or would he prefer to say that he cannot measure it at all?

Dr Hale: I do not think that I can go into greater analysis I agree, on looking back, that there are many things that one would like to have done, such as giving a placebo But I think with the condition existing, that would have been quite impossible because of the emotional atmosphere

Dr Sabin. Also, placebo would not have helped very much because the vaccinated and the non-vaccinated children also contracted some Type 2 infection, as Dr Hale has shown So the placebo approach does not lend itself to analysis here

But there is one point I would like to make which is contained in the orginal report, namely that in the Type 1 cases that did occur in the vaccinated children, there is an interval of about 10 to 20 days an interval of time in which the greatest multiplication of Type 2 might have been expected and when no Type 1 cases occurred; this is in line with the suggestion that that is a period during which perhaps the greatest effect of interference might have occurred

There are two possible effects, one by interference, immediately after extensive multiplication of the virus, and the other by this partially protective effect that several people have now demonstrated both experimentally and epidemiologically

Dr Payne I would like to ask Dr Hale whether there was any sign that the epidemic was particularly heavy in any specific area of the island and whether there was any sign that the vaccine distribution on the island differed at all from that?

Dr Hale: There was no sign that the epidemic was greater in any particular area. To a great extent, it was that the number of cases were largely dependent upon the population figures of that area, and vaccination was spread pretty widely throughout the island

Dr. Bodian: I am afraid that Dr. Hale did not answer my question I think that he is better aware of the circumstances of this study than anybody else in this room, and therefore I would like to get his frank opinion concerning the force, if any, of the protective effect

It seems to me that he is the one who has to make some sort of assessment, as quantitative as possible, of the force of an assumed protective effect, because obviously there have been variables unmeasured and unmeasurable.

Dr Hale Well, my opinion is that there was a protective effect in the vaccinated group

Dr Gear: I would like to ask Professor Hale if the virus isolated from the one Type 2 case is still extant, and secondly, if that case had any brothers or sisters, from whom specimens were taken for examination?

Dr Hale: I believe the strain is still extant

Chairman Stuart Harris: I would like to make a comment here in relation to Dr Bodian's remarks

Dr Hale presented his results a month ago at a meeting of some of the people in England interested in live poliovirus vaccines, and we did discuss the question of the evaluation of the protection The manner in which the feeding was done was over a period of some weeks after the epidemic had begun, and the statisticians present at the meeting felt that it was almost impossible to take any cases occurring in the non-vaccinated groups, in comparing those with the numbers in the vaccinated groups, until after the period of time when all these administrations of vaccine had been completed

That is why I think Dr Hale commented that after that period there were 13 further cases in non-vaccinated children, at which time the two groups should have been capable of being compared

Dr. Langmuir I appreciate and respect Dr. Hale's reluctance even to give an estimate of the magnitude of the effect, and I would like to mention a comparable situation in Chicago in 1956, where there was a very severe epidemic It was sharply concentrated in certain ethnic groups, particularly the Negro population We had a total of over a thousand cases, roughly 70 per cent paralytic We put the full forces of our epidemiological team into the situation Also, Dr Bundensen, the City Health Officer, put the full forces of his department behind it A million and seven hundred and fifty thousand doses of Salk vaccine were administered on almost a street-corner basis in a period of approximately one month to six weeks

We made every effort to try to evaluate whether this massive immunization program was effective, and I think Dr Gaylord Anderson contributed to an effort of this type We were forced to conclude that it was totally impossible to draw any conclusions as to whether this was effective in any way in this epidemic There was a symmetrical curve up and down It did turn over early in the year, compared to most epidemics, at the end of July instead of early September, but this was not proof that the vaccine had affected the epidemic Also, for the record there were approximately 250 cases of disease, mostly paralytic, developing in recently vaccinated children This did not cause any hysteria or any problem in the population They accepted this as a vaccination given too late

Dr. Anderson: Confirming what Dr Langmuir said about the Chicago outbreak, as close as it could be studied, we could find no evidence that it differed at all from earlier outbreaks in the same city, both in terms of magnitude and in terms of shape of epidemic curve

It did end earlier than usual On the other hand, it began much earlier, so that one could not see any demonstrable effect of this mass immunization with the Salk vaccine

Dr Dick I would like to introduce a rather difficult problem into this assessment I do not want this to be in any way misinterpreted but in any population group there are a number of *children* who die from natural causes And I would like to know from Dr Hale how many of his vaccinated children died in the one-month period after vaccination or in the six month period after vaccination. I am not implying that these deaths had anything to do with vaccination whatsoever I want to stress this—the deaths have absolutely nothing to do with his giving vaccine, but are they considered in his surveillance?

What number of children, roughly, would he expect to die in a group of 200,000 children in Singapore in one month? Furthermore, can the mortality in his vaccinated group and non vaccinated group be used to show that the groups are comparable?

Dr Hale: As indicated in the infant mortality figures, for example, a couple of children in the vaccinated group were killed in road accidents. Obviously, they are going to die of all sorts of causes

Dr Bodian It just occurred to me in connection with what Dr Dick said, and considering the possibility of future epidemics where one might want to carry on and learn from this experience, that it is possible to study the comparability of the two groups, the vaccinated and the unvaccinated, by studying the number of deaths, for example, and causes of death in two groups such as yours, would it have been possible to determine the comparability?

If the groups are not comparable, it is obvious that we can give very little weight to the difference in the attack rate even to the 13 late cases

Chairman Stuart-Harris Dr. Hale, do you have anything to add to that?

Dr Hale I have not done that, but I suppose we could go back and investigate the tests in the two groups

Dr. Payne I think what Dr Bodian suggested is extremely valuable I would like to carry it further and suggest that this study should not be confined to deaths, but extend to other recognized diagnosed illnesses We have, in fact, used this in various control trials of other vaccines, as a measure of comparability of the two groups, even when we had a placebo

Dr Sabin. May I add that Professor Smoro-

dintsev, in samples of about 7,500 vaccinated and 3,500 non vaccinated children, was looking for all sorts of other illnesses quite unrelated; and the data as I have seen them, showed the rate per ten thousand very close and comparable for the areas in which they worked So that this can be used as a means of checking on the selection or the sampling

DR SOPER: The question which was raised earlier regarding the approach of the public health officer and the scientist, possibly merits a little further comment I am not going to make that comment, but I do want to point out that in the work which will be reported later this week by others who did the work, an opposite approach was taken And in Colombia, where the virus had been identified as a Type 1 virus, Type 1 virus was taken into the community for vaccination In Nicaragua, where the outbreak was with Type 2 virus, Type 2 virus was taken in and given initially during the epidemics

DR BARR The only question I have is Would a study of the groups that you immunized with Type 2 for Type 1 antibodies, as compared with the other group at this time, show you whether or not both groups are equally protected, and whether or not you can give any lead on what the Type 2 may have done in giving you some protection during this period of time?

DR HALE I do not think that would be of very much help, because I expect that one would have to have two groups, each of about comparable size, one of 200 000 and another just under 300,000 I would expect that the Type 1 antibody distribution would be approximately the same

DR SABIN I have fed Type 1 Mahoney virus to 29 cynomolgus monkeys that had had Type 2 virus previously The incidence of paralysis among 40 monkeys that received only the Mahoney virus and that had no previous Type 2 was 60 to 70 per cent In the monkeys that had previous Type 2 infection, it was about 20 per cent The figures are on record, I do not remember them But in any event, all the Type 2 developed Type 1 antibody So, the difference is in the incidence of paralysis, not in the incidence of infection

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Dr Sabin: May I add that Professor Smoro

THIRD SESSION

WEDNESDAY, 24 JUNE 1959

Chairman

DR JAMES H GEAR

Director of Research

Poliomyelitis Research Foundation

Johannesburg, Union of South Africa

TOPIC III. PROPERTIES AND BEHAVIOR OF ORALLY ADMINISTERED ATTENUATED STRAINS *(continuation)*

Presentation of Paper by:

Prof A A Smorodintsev

(DISCUSSION)

TOPIC V. FIELD TRIALS

Presentation of Papers by:

Dr Charles H Stuart Harris

(DISCUSSION)

Dr Sven Gard

Dr J D Verlinde

(DISCUSSION)

Dr Robert N Barr and

Dr Herman Kleinman

Dr Henry Bauer

(DISCUSSION)

Dr A J Lebrun

Dr Stanley A Plotkin

(DISCUSSION)



TOPIC III. PROPERTIES AND BEHAVIOR OF ORALLY ADMINISTERED ATTENUATED STRAINS
(continuation)

4. EXPERIMENTAL AND EPIDEMIOLOGICAL DATA ON THE EFFECTIVENESS OF LIVE POLIOMYELITIS VACCINE

Part 1. Experience in the Production, Biological Control, and Use of Live Poliomyelitis Vaccine Made from Sabin Strains

A. A. Smorodintsev, A. I. Drobyshevskaya, V. I. Ilyenko, T. E. Klyuchareva, and O. M. Chalkina

Department of Virology, Institute of Experimental Medicine of the USSR Academy of Medical Sciences, Leningrad, USSR

Dr Smorodintsev (*presenting the paper*) In the period 1957-1959 the staff of the Virology Department of the Institute of Experimental Medicine, in co-operation with the Leningrad City Department of Health, the nervous diseases clinic of the Leningrad Institute of Medical Pediatry, the infectious diseases clinic of the Leningrad Institute of Sanitation and Hygiene and the Ministries of Health of the Latvian, Byelorussian, and Moldavian SSR, studied the reaction causing and immunogenic properties of the live poliomyelitis vaccine produced in Leningrad from attenuated strains of Types 1, 2, and 3 of the poliovirus

The work of the Virology Department began in the middle of 1956 with the study, in experiments on monkeys, of the pathogenic properties of the attenuated strains of poliovirus received from Professor A Sabin of the United States of America

In 1957 detailed laboratory and clinical observations were carried out on a group of 150 infants in Leningrad, followed by a further contingent of 2,500 children of pre-school age in 1958 These investigations showed the harmlessness of the live vaccine for the most susceptible group of vaccinated children and children in contact with them and also proved the absence of reversion in the vaccinal strains after prolonged passage through the intestinal canal

On this basis we obtained, in September-October 1958, the permission of the Sera and Vaccines Commission and the Collegium of the USSR Ministry of Health for the immunization of 20,000 children, which took place in the Latvian SSR in January-March 1959

In view of the harmlessness and immunological effectiveness of the live vaccine, the USSR Ministry of Health, in March 1959, gave permission for a considerable expansion in the volume of observations, which made it possible for us to immunize in April-May 1959 about 1,500,000 children in the Latvian, Byelorussian and Moldavian SSR and to study, in 1959, the epidemiological effectiveness of the live vaccine

In the period 1957-1958 the authors studied the technological process of producing the live poliomyelitis vaccine from the attenuated Sabin strains, the methods of laboratory control for harmlessness and the technique of practical use of the live vaccine on about two million children

Below will be found the results of the observations carried out

1 *The Strains of Virus Used and the Series of Live Vaccine*

To study the live vaccine three different series

four to six liters of vaccine from these passage strains of various types This product (Series 1) showed an activity for tissue cultures of 6 5-7.2 log 10 and was used after detailed control tests for carrying out the first stage of observations on a group of vaccinated and contact children totalling about 30 000.

(2) Series 2 of the vaccine was made between September 1958 and March 1959 from the latest Sabin strains, obtained from the author in 1957, and used by him for preparing in the United States of America a series of live vaccine sufficient for two million vaccinations (20 liters of each type: Type 1, LSc 2 ab; Type 2, P 712, ch 2 ab; and Type 3, Leon 12 ab).

In contrast to the strains used earlier, these viruses were isolated in the form of pure lines by the Dulbecco method and were of minimum pathogenicity for monkeys and genetically more homogeneous than previous strains

In order to achieve maximum uniformity and comparability in the live vaccine used abroad and in the USSR, we prepared for mass observations 23 liters of each type of vaccine from the latest standards of attenuated Sabin strains, the biological titer of which was equal to 7 0 log 10 for all three types The vaccine of Series 2 was used for mass immunization of 1,800 000 children from six months to 14-18 years of age

(3) We received the original preparation of the Sabin live vaccine, which he had distributed to various countries for scientific study of its effectiveness, from the author and used about 50,000 doses for observations This same product was used as standard vaccinal material for the production of the vaccine in Series 2 (Table 2)

TABLE 2 INTRACEREBRAL AND SPINAL ACTIVITY IN MACACA RHESUS MONKEYS OF VARIOUS SERIES OF LIVE POLIOMYELITIS VACCINE

TYPE OF VACCINE	SOURCE OF VACCINE	QUANTITY IN LITERS	LOG $TCID_{50}$	IN THE THALAMI	IN THE SPINAL MARROW
1	Sabin 1958	1	7 2 6 2	0/3	0/6 0/6
2	Sabin 1958	1	7 0 6 0	0/3	0/6 0/6
3	Sabin 1958	1	7 0 6 0	0/3	0/6 0/6
1	I E M Series 1 1957	6	7 0 6 0 5 0	0/6	1/6 0/6 0/6
2	I E M Series 1 1957	4	6 5 5 5 4 5	0/6	1/6 0/6 0/6
3	I E M Series 1 1957	4	6 5 5 5 4 5	0/6	0/6 0/6 0/6
1	I E M Series 2 1958	23	7 0 6 0 5 0	0/10	0/5 0/5 0/5
2	I E M Series 2 1958	23	7 0 6 0 5 0	0/10	0/5 0/5 0/5
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of the product, made from the Sabin strains, were tested

(1) The first series of vaccines was produced at the beginning of 1957 in the Virology Department of the Institute of Experimental Medicine in Leningrad from the variants we had obtained of the following Sabin strains of 1955-1956.

Type 1 LSc Cincinnati of 22 December 1955
Type 2 P 712 - 10 av of 5 April 1956
Type 3 Leon 14 av of 5 April 1956

In view of the instability of the neurotropic properties of these strains, as apparent from Sabin's observations, and the intensification of those properties during the process of multiplication in the intestinal canal of vaccinated volunteers, we subjected the strains to a further 24-30 passages through monkey kidney tissue monolayer cultures according to the schedule used by Sabin for his primary selection of attenuated variants of the poliomyelitis virus For this, each successive passage was carried out by *inoculating two test-tubes with a massive dose* of virus taken in a volume of 10 ml from a Roux bottle with an area of 60 square millimeters portions were transferred into separate Roux bottles

After three days incubation, bottles were selected with developing cytopathogenic changes corresponding to the minimum dose of the inoculum and two test-tubes were again given a massive inoculation. This procedure was repeated up to 24 or 30 times for each serotype of the vaccinal viruses.

The final specimens of multiple-passaged cultures were tested for activity on Macaca rhesus monkeys by intracerebral and intraspinal infection In doing this we aimed at a further attenuation of the neurotropic activity of the vaccinal strains and also on greater stabilization of their inherited properties in relation to the intensity of reversion on administration of the live vaccine to susceptible children

As will be seen from Table 1, the live vaccines obtained from different variants of the Sabin vaccinal strains did not produce pathological reac*tions in Macaca rhesus monkeys on intracerebral* injection, and caused about 10 per cent paralysis in the case of Types 1 and 2 on intraspinal injec-

TABLE 1 PATHOGENICITY FOR MACACA RHESUS MONKEYS OF VACCINAL STRAINS OF POLIOMYELITIS VIRUS INJECTED INTO THE BRAIN OR THE SPINAL MARROW, IN THEIR INITIAL STATE AND AFTER 2-4 PASSAGES THROUGH THE INTESTINAL CANAL OF SUSCEPTIBLE CHILDREN

UNDILUTED TISSUE CULTURE	METHOD OF INJECTION	TYPES OF VIRUS		
		1	2	3
Sabin's original vaccine strains	Intracerebral	0/12	0/12	0/12
	Intraspinal	3/15	2/24	0/29
I E M (Leningrad) live vaccine	Intracerebral	0/10	0/10	0/10
	Intraspinal	1/10	1/12	0/13

Numerator—Number of sick monkeys
Denominator—Number of monkeys in the group
I E M is the Institute of Experimental Medicine of the USSR Academy of Medical Sciences

After two hours contact of the virus with the tissue culture the surplus was discarded and it was mixed with fresh nutrient medium The inoculated test-tubes were transferred to the incubator *for two days*, the accumulated virus was diluted to 10^{-6}, 10^{-7}, and 10^{-8}, and 1 ml tion Similar indices of pathogenicity for monkeys were shown by the standard Sabin vaccine of 1958 which had no intracerebral activity and had only retained a slight neurotropic activity on intraspinal injection

At the beginning of 1957 we produced from

four to six liters of vaccine from these passage strains of various types This product (Series 1) showed an activity for tissue cultures of 6 5-7.2 log 10 and was used after detailed control tests for carrying out the first stage of observations on a group of vaccinated and contact children totalling about 30,000

(2) Series 2 of the vaccine was made between September 1958 and March 1959 from the latest Sabin strains, obtained from the author in 1957, and used by him for preparing in the United States of America a series of live vaccine sufficient for two million vaccinations (20 liters of each type: Type 1, LSc 2 ab, Type 2, P 712, ch 2 ab, and Type 3, Leon 12 ab).

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2. The live vaccine was produced in accordance with Sabin's technological recommendations under instructions we had worked out for the production and control of the live vaccine and which were confirmed by the Sera and Vaccines Commission of the USSR Ministry of Health in November 1958

The tissue cultures were prepared from the kidneys of monkeys who had shown negative findings in a tuberculosis test made a fortnight before the kidneys were removed On autopsy the presence of pathological changes (tuberculosis, ulcerative colitis characteristic of dysentery and parasitic disease) was checked The most satisfactory monkeys were from Viet Nam, while the weakest and most often infected with dysentery were those from India Animals from China occupied an intermediate position

The monkey kidneys prepared for trypsinization were brought into contact with trypsin for 18-20 hours in the cold (2° to 4°C) The tissue was kept constantly mixed by means of a magnetic stirrer and the cellular suspension obtained was centrifuged and suspended in a nutrient medium consisting of Hanks' solution, 2 per cent calf serum, 0 5 per cent hydrolysate of lactalbumen, and antibiotics (penicillin, streptomycin, mycostatin), The cells were transferred to Roux bottles made of Czechoslovakian neutral Sial glass with a capacity of 1,200 ml To reduce scrap we trypsinized and cultivated cells separately for each monkey

The Roux bottles were firmly stoppered with rubber stoppers and kept at 36° to 37°C until a solid layer of kidney epithelium had formed, this happening by the eighth day During this time the medium had been changed once (after four days). After observation under a microscope with a magnification of 150x, bottles were selected and infected which contained faultless monolayer cultures

Ten per cent of the Roux bottles for each monkey were left uninoculated and were kept under observation for three weeks (liquid medium being changed once every 7-10 days) in order to detect spontaneous cytopathogenic Simian viruses

We removed the old medium from the Roux bottles allocated for cultivation of the virus by means of a siphon under vacuum and the cellular mass was again washed with Hanks' solution Fresh medium was prepared from Earle's solution with the addition of 0 5 per cent of hydrolysate of lactalbumen and antibiotics with a pH of 7.5-7.6 After careful mixing the original Sabin vaccine of the corresponding serotype was introduced into the medium in the quantity of 1 ml from a dilution of 1:100 to 1 liter of medium (100 TCID per 1 ml. of medium). After mixing, the medium obtained together with the virus was dispensed by siphon into the prepared Roux bottles containing tissue culture, at the rate of 120 ml of liquid per bottle. The inoculated bottles were placed in the incubator at 36°-37°C

Three days after introduction of the virus the infected bottles were inspected, and if the cells had completely degenerated the virus containing liquid was collected by siphon and put into three liter bottles, separately for the cells from each monkey, this corresponding to 1.5-3 liters of vaccine

These materials were frozen and stored at t° −12°C until the end of the control tests for sterility, biological activity and harmlessness

On completion of the control tests the seed suspensions of virus obtained after cultivation in kidney cultures from different monkeys were unfrozen in a refrigerator at a temperature of 2° to 4°C After 24 hours of standing the clarified virus containing liquid was drawn off into a general container through a close cotton-wool filter We did not undertake any other additional filtration, such as through asbestos or other solid filters, considering that for a live vaccine which has passed all tests this not only has no point but may even lead to a reduction in its biological activity, i e, to a worsening of the quality of the product We therefore confined ourselves to clarifying the vaccine through a close cotton-wool filter, passing through it not more than 5 liters of the separate seed suspensions

3 *Biological Control*

To test the live vaccine for sterility, biological activity and harmlessness, the necessary tests were carried out in the following order

(a) preliminary control of sterility and biological activity of the seed suspensions obtained from cultures of the kidneys from individual monkeys in a volume of 1 5-3 liters,

(b) a test for harmlessness of the pooled seed suspensions which had proved sterile and highly active in virus content, these tests were set up

with material pooled from various samples taken from the individual seed suspensions which had been tested for sterility and biological activity;

(c) final control for sterility and biological activity of the vaccine pooled according to individual serotypes

(a) *Test for sterility and biological activity*

From each flask containing a seed suspension of virus obtained from the kidneys of one monkey a 100 ml sample was taken to test these materials for sterility and the infective titer of the virus After this the remaining virus suspension was frozen at −12°C and kept in that state until the vaccine of the type concerned was pooled

The sterility test was carried out by inoculating the vaccine on sugar bouillon and Sabouraud's medium The results of this inoculation were calculated after eight days' incubation on the bouillon at 37°C and 22°C and on the Sabouraud's medium at 37°C or 22°C From 1500 to 3000 ml of virus-containing fluid were obtained from each monkey

At the same time seed suspensions of virus of various types obtained from each monkey were tested for virus content by inoculating homotypic cultures of kidney epithelium (two parallel series) with various ten fold dilutions of Medium No 199 The results of the test for biological activity of the seed suspensions were read on the fourth to the seventh day after inoculation

Side by side with the titration of the biological activity of the virus its specificity was tested by culture of the starting material in the presence of specific serum of homologous type Complete absence of any virus growth in such test-tubes showed its specificity

(b) *Test for harmlessness*

After completing the test for harmlessness and biological activity of all the individual virus pools from the various monkeys, we pooled six or seven test specimens of the seed suspension

For the test for presence of the tuberculosis bacillus we used the sediment obtained after 30 minutes centrifugation at 4 000 to 6,000 r p m of 400 ml of virus-containing liquid of each type. The sediment was suspended in 20 ml of physiological solution and injected intraperitoneally in doses of four milliliters each into four guinea-pigs, which were kept under observation for two months After this period the animals were sacrificed and the autopsy was carried out in the presence of a specialist (M A Linnikov). In not one instance was tuberculosis found to have developed in the guinea pigs into which the sediment from the seed suspensions of monovalent suspensions of the three types of virus, re-suspended in physiological solution, had been injected

There was no finding of virus B on injection of each sample in 1 ml intracutaneous doses into six rabbits—0 1 ml. in each of 10 sites—and subcutaneously in a dose of 9 ml. No rabbits died and no skin changes were noted in 21 days

Negative results were also obtained in the test for the presence of lymphocytic choriomeningitis on intracerebral infection of 20 white mice with two consecutive passages

No Coxsackie viruses were found on infection of one-day old and two-day old suckling mice (Table 3)

On observation of control bottles containing cultures of kidney cells uninfected with poliomyelitis viruses, we found on rare occasions cytopathogenic changes in the cells on the seventh and eighth day after the change of medium, i e, on the eleventh and thirteenth day after culture of the trypsinized cells. The degeneration noted did not differ from the changes in the infected bottles The serum against the three types of poliomyelitis did not eliminate the cytopathogenic effect, which was maintained on passage The suspensions connected with these bottles were discarded In the majority of the uninfected control bottles the culture was well maintained without degeneration for a month or more

Foamy viruses were discovered on one occasion in Roux bottles containing kidney epithelium monolayer cultures, on the sixth day after culture of the trypsinized cells, and had to be eliminated

(c) *Final test*

The vaccine of various serotypes pooled in the series was again tested for sterility by the method outlined above A final test of virus content in the monovalent suspensions was also carried out by infecting kidney epithelium monolayer test-tube cultures with various ten-fold dilutions of centrifuged virus suspension on synthetic Medium No 199. The results were read on the fourth to the seventh day after infection

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The absence of extraneous viruses in the monovalent suspensions was tested by sowing in ten test-tubes, containing a monolayer culture of simian renal tissue, equal volumes of the mixture of the poliomyelitis virus being tested and homologous immune rabbit serum with a titer of virus-neutralizing antibodies of 1 4 000 1:16,000, diluted 1 5 with Earle's solution After six days the solution was discarded and a fresh medium substituted containing 20 per cent hyperimmune serum. The development of the cytopathogenic effect caused by extraneous viruses was checked for 12 days in the presence of a neutral mixture of poliomyelitis virus and antibodies

After tests for sterility, harmlessness, biological activity and type identification of the virus in separate samples, we pooled the seed suspensions according to type after unfreezing them in the refrigerator at 4°C for 48 hours For this purpose we used a flask with three siphons one of them, which had an enlargement tightly filled with cotton-wool, was used for drawing off and simultaneously filtering, the second was connected with an automatic syringe for dispensing the vaccine into ampoules, and the third was connected with a vacuum pump (in pooling the liquid from the flask) or with a Richardson bulb (on dispensing the vaccine)

During dispensing, inoculations for sterility were made at the beginning, in the middle and at the end and a sample was taken for titration of the biological activity of the virus, for its identification and for testing infectivity of the vaccine in experiments on monkeys

The vaccine was tested for harmlessness on monkeys by injection into the cerebral thalami The skull was trepanned at a distance of 6 millimeters from the center line along the coronary suture The virus was injected by means of a No 24 needle, through the trepanned opening at a depth of 2 5 centimeters, in a dose of 0 5 ml, from one side

Injection into the spinal medulla was carried out by injection of 0 1 ml of vaccine in dilutions of 1 1, 1 10 and 1 100, which corresponded to 1,000,000, 100,000 and 10,000 $TCID_{50}$, respectively, in the lumbar enlargement of the medulla The virus was injected into the fourth intervertebral space counting upwards from the iliac crest, this corresponding to the first intervertebral space lying below the twelfth thoracic vertebra

The virus was injected by means of a 1 5-centimeter long No 20 needle somewhat to the side of the center line Proof that it had been successfully injected into the lumbar enlargement was the fibrillary contraction of various muscle groups in the hind extremities and histopathological examination of the monkeys, which were sacrificed on the twenty first day after injection of the vaccine The presence of a scar was looked for in the area where the needle was inserted in the grey matter of the anterior horns of the lumbar enlargement, examined at 20 different levels at intervals of 1 millimeter. The histopathological changes present in this area and in the cervical section of the spinal medulla were recorded (10 levels at 1 millimeter intervals)

4 *Technique of Use of the Live Vaccine*

The vaccine is exceptionally stable if it is stored in the frozen state at a temperature of −12 to −15° In these conditions its biological titer will remain unchanged for one or two years

For practical use we diluted the initial vaccine, which had a biological titer of 7 0 log 10 and over, ten fold or hundred-fold and used it for oral administration in doses of 0 1 ml. (two drops from an eye dropper) or 1 ml (through a syringe of 1 or 2 ml, capacity) respectively

The dilute vaccine must be transported and stored in a thermos with ice at a temperature of 2°-4°C, which maintains its biological activity for three to seven days.

The best way of using the live vaccine is to issue the ready-for use product in a dilution of 1 10, corresponding to a content of 100,000 tissue infective doses in 0 1 ml of the diluted preparation The 10 ml penicillin flasks containing ready for-use vaccine of Types 1, 2, and 3, frozen in CO_2 or in an ultra-refrigerator, are placed in a thermos, where they slowly unfreeze and make it possible to use the cold product for one or two days without the addition of ice

Two different schedules of administration have been used for live vaccine.

(a) consecutive administration of monovalent vaccines of Types 1, 3, and 2 (the Sabin method) at intervals of three to four weeks,

(b) the administration of the vaccine in two stages to start with Type 1 and then, after a

Table 3 Control Data on the Live Poliomyelitis Vaccine Series 2 Issued in 1959

Types of Vaccine	Sterility						Harmlessness				Biological Activity in Log $TCID_{50}$
	Sugar Bouillon		Tarozzi's Medium		Sabouraud's Medium		Virus B (Rabbits)	Lymphocytic Chorio-meningitis (White Mice)	Coxsackie (Suckling Mice)	Mycobacterium Tuberculosis (Guinea-Pigs)	
	22°	37°	22°	37°	22°	37°					
1 LSc 2 av		Sterile					0/15	0/60	0/26	0/15	7 0
2 P 712 ch 2 av		Sterile					0/15	0/60	0/26	0/15	7 0
3 Leon 12 av		Sterile					0/15	0/60	0/26	0/15	7 0

Key. Numerator, number of sick animals
Denominator number of animals in the test

Each time we administered a full inoculative dose of each serotype (100,000 units).

For virological examination we collected fecal specimens from inoculated children in sterile penicillin flasks (15 ml) which were rubber-stoppered and taken to the laboratory in a thermos with ice. The feces were stored until examination in the frozen state (−15°C to −20°C) for not more than 7-14 days. Before examination the feces were unfrozen and a 20 per cent suspension by weight was prepared in Hanks' solution and centrifuged for 30 minutes at 5,000 r p m. The transparent supernatant fluid was combined in the cold with an equal volume of antibiotics containing 2 000 units of penicillin and 2 000 γ of streptomycin per 1 ml. After one hour, monkey-kidney monolayer tissue *cultures or human embryo fibroblasts were in-*fected with a suspension of feces to a pH of 7 4. Preliminary examinations showed that human fibroblasts have quite a high sensitivity to poliovirus, this sensitivity differed from that of the monkey kidney cells in parallel titration of virus activity in these cultures by not more than 0 5 log 10. It must be kept in mind, however, that monolayer cultures of kidney epithelium from Macaca rhesus monkeys are less sensitive than similar cultures from Macaca cynomolgus monkeys, the difference being expressed for Type 1 by a factor of about 0 7 log 10. Thus, the actual virus content of the feces is somewhat diminished if cells from Macaca rhesus monkeys, which are more easily available in our conditions, are used for titration.

For the titration of virus in fecal specimens test tubes were selected with a solid layer of normal cells. The supernatant suspension of feces was diluted tenfold in Mixture No 199 (from 10^{-1} to 10^{-7}) and each dilution was used to infect material in two test tubes, which were examined after one hour, one day, four days and 8 10 days, after which the experiment was considered finished. Test tubes showing degeneration developing after one hour of infection were excluded from the experiment and a corresponding portion of feces was examined in larger dilutions, or, if degeneration had progressed a long way, the examination was repeated. If degeneration appeared after one day the culture fluids were transferred from all test tubes showing degenerative changes to test tubes containing fresh tissue. The specificity of the cellular degeneration developing in the last test tubes was tested by additional passage and a parallel neutralization test was carried out with immune sera of homologous types.

Our material covers observations on quantitative changes in the vaccinal strains in 352 children, of whom one-third were examined after consecutive vaccination with the various serotypes and two-thirds were immunized with one of the serotypes. From each infant 10-12 fecal specimens were taken after 1, 4, 7, and 14 days and then at further intervals of 7 days up to 63-70 days after vaccination.

In examination, during two years, feces from 352 children taken before vaccination, 69 different cytopathogenic viruses were isolated, of which 48 belonged to the ECHO group, 14 to the Coxsackie group and 7 were polioviruses. The vaccinated or contact group of children were serologically examined in order to titrate the virus neutralizing antibodies in several serum specimens taken before vaccination and at various periods after immunization. Some of the children were examined several times at intervals of 3 4 months for as long as 1½ years after vaccination and some were examined two or three times in order to study the intensity of growth in the number of antibodies 3, 8, and 16 weeks after immunization.

The sera were heated at 56°C and kept in the frozen state until they could be examined. Sera collected before immunization, and at various lengths of time after it, were tested in a single neutralization color test (pH test) in the presence of 100 TID_{50} of virus of each type. The bulk of the observations were carried out with monkey kidney cells, the rest with trypsinized human embryo tissue fibroblasts.

I. Quantitative Changes in the Multiplication of Attenuated Strains in the Intestinal Canal of Susceptible Children

A study of what happens to vaccinal strains of poliovirus has shown that they consolidate and multiply well in the intestines of the overwhelming majority of inoculated children without antibodies to the poliovirus in their blood, and reach considerable concentrations. The virus is usually found in the intestinal contents

month, final immunization with a divaccine of Types 2 and 3. In these conditions the percentage of positive reactions for Type 1 reaches 95 per cent in children with no antibodies to Type 1, 80 per cent for Type 2, and 75 per cent for Type 3. If the duration of immunity under this schedule turns out to be insufficient it will be advisable after one year to carry out a final immunization with a polyvalent vaccine of Types 1, 2, and 3.

Conclusions

1. The use of attenuated Sabin strains makes it possible to obtain in monolayer cultures of Macaca rhesus monkey kidneys a product of high infective titer free from extraneous bacteria and viruses.

2. The live vaccine prepared in Leningrad in two series of five to twenty-three liters for each type has been marked by high stability during lengthy storage and has kept its biological activity after unfreezing and ten-fold or hundred-fold dilution at a temperature of 2°C-4°C for up to seven days.

3. The authors used for mass immunization in the USSR a schedule of separate administration of the three monovalent vaccines for Types 1, 3, and 2 at monthly intervals in that order, and also a quicker, and in practice, more convenient schedule of two stage immunization (Type 1, followed after a month by a divaccine of Types 2 and 3).

Part 2. Virological and Immunological Characteristics of Vaccinal Infection in Children Inoculated Per Os with a Live Poliomyelitis Vaccine Made from the Sabin Strains

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In our study of the safeness of live poliomyelitis vaccine made from attenuated Sabin strains in Leningrad infants' homes on children aged six months to three years who had not been inoculated with the Salk killed vaccine, we had among these children a large section (up to 70 per cent) of persons of maximum susceptibility with no antibodies to any type of poliovirus. This is due to the fairly complete isolation of these groups from contact with any other children and with adults. The children under our observation were in constant and fairly close contact with each other and also with the staff of adults serving them. They were under constant medical observation by local staff and by our consultant neuropathologists and pediatricians.

In the course of 1957-1958 the following questions were studied in detail:

1. The quantitative dynamics of multiplication of the vaccinal strains of the poliovirus in the intestinal canal of vaccinated children.
2. The spread of the attenuated strains from vaccinated children to contact groups of unvaccinated children.
3. The intensity and duration of immunological changes in children inoculated with live vaccine (under various immunization schedules) and in contact groups.

Materials and Methods

We carried out vaccination perorally by the administration of 100,000 tissue cytopathogenic doses of vaccinal strains of Types 1, 2, or 3 in 5 ml of cooled boiled milk. In a number of experiments different immunization schedules were also tried out, such as a double inoculation schedule (administration of Type 1 followed after a month by administration of a divaccine of Types 2 and 3) or a single immunization with a trivaccine of Types 1, 2, and 3.

curves indicate quite large fluctuations in the intensity of multiplication of the virus in different children and show that there is no great difference in this respect between Types 1, 2, and 3 Study of the individual curves of virus multiplication among more than 300 vaccinated children makes it possible to classify virus reproduction into four different types (Fig 2):

than Type 2, and the least active reproduction is noted in virus of Type 3 (Fig 1).

We did not find any essential difference in the quantitative changes in titer of the vaccinal strains of Types 1, 2, and 3 of the virus whether they were administered to triply negative children in doses of 100,000 or of 10,000 units At the same time, a sharp drop was noted in

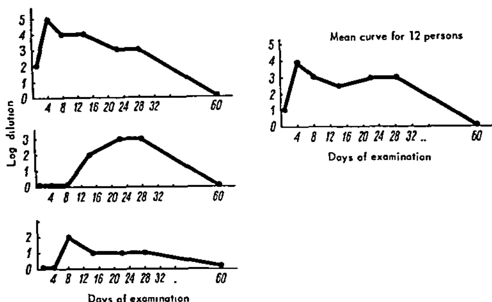


Fig 2 Course of multiplication of virus of Type 2 in the intestinal canal of children inoculated with live vaccine

1 Intense multiplication of the virus in the first 14-23 days with a maximum concentration of as much as 10^6 and 10^7 $TCID_{50}$ per gram of feces with subsequent reduction in the number of viruses after 30-50 days.
2 Moderate multiplication of the virus (titers not exceeding 10^3 and 10^5 $TCID_{50}$ per gram of feces)
3 Poor and irregular multiplication of the virus (titers 10^1 and 10^2 $TCID_{50}$ per gram of feces)
4 Negative curves with no excretion of the virus on examination at any stage

All three types of vaccinal strains multiply quite intensively in the intestinal canal of children aged 6 months to 3 years Virus of Type 1, however, is excreted in somewhat higher titers

the titers of virus multiplication on administration of a smaller dose of virus and this was accompanied also by weakening in the immunological response This shows the existence of a solid reserve in the immunogenic activity of the dose of 100,000 infective units recommended by Sabin This reserve would not be substantially weakened by ten-fold reduction but would be insufficient if the dose were reduced one hundredfold.

The correlation between the intensity of multiplication of attenuated strains of the poliovirus and the accumulation of antibodies in the blood of inoculated children is scarcely noticeable among the groups with intense to moderate multiplication but is clearly marked in groups with minimum or negative reproduction. A good ac-

on the day following inoculation and reaches maximum titers on the 7th-14th day, followed by a gradual reduction up to the 28th day and subsequent excretion in small quantities. The mean duration of excretion of the virus is 30 50 days. The virus is rarely excreted after the 50th day, and then not regularly. Investigations have shown that the age of the child has no essential influence on the intensity of virus multiplication

Figure 1 shows individual curves for virus carriage in children vaccinated with live vaccine against poliomyelitis of Types 1, 2, and 3 These

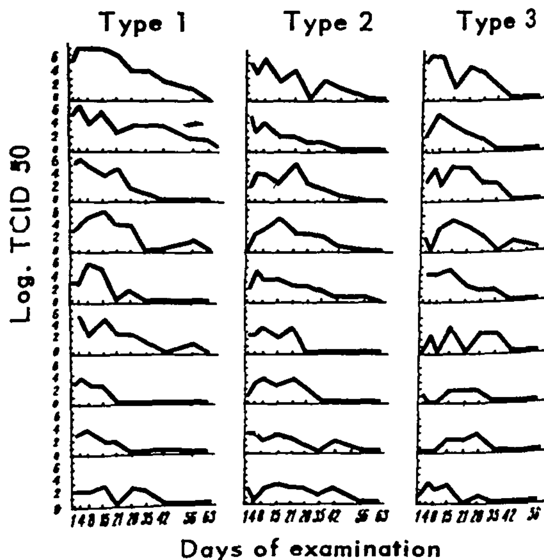


FIG 1 Course of multiplication of the poliomyelitis virus in the intestines of children inoculated with live attenuated vaccine of Types 1, 2, and 3. (Individual curves)

Figure 4 gives figures for the excretion of the introduced virus by children in the contact group at various dates after the beginning of immunization and the total percentage of children involved in contact virus carriage during those periods.

The greatest intensity of contact infection was noted in the case of Type 2, where virus carriage reached 80 per cent after 130 days' observation Rather smaller indices were noted for Types 3 and 4

It is interesting to note that the greatest development of virus carriage was observed between the 10th and 50th days, when the cumulative curve showing infected children among the contact group had almost reached its peak, thereafter showing little change Apparently this is explicable by the concurrent increase in the multiplication rate and excretion of the virus in inoculated children, who infected the environment with the maximum quantities of virus in the period between the 7th and 21st days after vaccination

Table 4 gives titers in individual children at different periods after immunization The highest figures are concentrated between the 20th and 40th days and the duration of virus carriage fluctuates from less than 10 to 70 days

TABLE 4 DURATION OF VIRUS CARRIAGE AND CONCENTRATION OF POLIOMYELITIS VIRUS IN THE INTESTINAL CANAL OF CHILDREN IN CONTACT WITH INOCULATED CHILDREN IN INFANTS' HOMES

	before	10	20	30	40	50	60	70	80	90	Duration of virus carriage in days
Type 1	0	2	4	1	1	0	0	0	0	0	40
	0	0	2	4	2	2	1	0	0	0	50
	0	0	2	0	2	3	0	0	0	0	40
	0	0	0	1	3	1	0	0	0	0	30
	0	0	0	0	4	2	0	0	0	0	20
Type 2	0	0	2	5	3	1	1	0	0	0	50
	0	0	0	2	4	0	0	0	0	0	20
Type 3	0	1	3	3	0	0	0	0	0	0	30
	0	0	3	2	1	1	1	1	1	0	70
	0	0	2	5	4	3	0	0	0	0	40
	0	0	3	4	0	0	0	0	0	0	20
	0	0	0	4	0	0	0	0	0	0	10

Time of examination in days after introduction of the virus into the community

Table 5 shows data for selectively examined children in the contact group from two infants' homes in which vaccination with virus of Types 1 and 3 had been carried out Of the 19 children with low initial antibody titers (0 or 1 4) in contact with virus of Type 1, ten acquired antibodies Of the 22 children of a similar group in contact with virus of Type 3, 17 acquired antibodies, i e., the overwhelming majority of those examined

TABLE 5 QUANTITATIVE CHANGES IN ANTIBODY TITER IN CHILDREN AGED 1 TO 3 YEARS IN CONTACT WITH CHILDREN INOCULATED WITH VACCINES OF TYPES 1 OR 3

Antibody Titer 4 Months After the Beginning of Contact	Infants' Home No 1 (Vaccine of Type 1) 28 Children						Infants' Home No 3 (Vaccine of Type 3) 40 Children					
1024				1	2	2					1	
256					1		1			2	2	1
64	7			1			3		1	8		
16	1	2	2				8	1	3			
4		3					4	4				
0	6						1					
No of children	14	5	2	2	3	2	17	5	4	10	3	1
Antibody titer before contact	0	4	16	64	256	1024	0	4	16	64	256	1024

cumulation of virus in the intestines of the inoculated child is always accompanied by an intensive rise in the antibody titer, a rise which is inhibited or absent where the virus multiplies at a lower rate in the intestinal canal Figure 3 shows indices of reproduction of virus of Type 1 in individual children and the changes in antibody titer in their blood

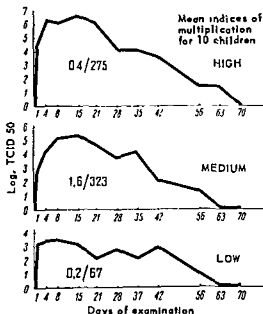


Fig 3 Correlation between the intensity of multiplication of vaccinal poliomyelitis virus of Type 1 in the intestinal canal of inoculated children and the number of antibodies 90 days after immunization

II The Spread of Vaccinal Strains from Inoculated Children to Contact Groups

The intensive reproduction of vaccinal strains in the intestinal canal of inoculated children noted above and the consequent excretion of the virus into the environment can lead in conditions of close and direct contact such as exist among children in the first years of life, to extensive spread of the vaccinal virus to the contact groups

We studied this question in three infants' homes on children aged 1-3 years, of whom between 30 and 50 per cent had received a single administration of 100,000 units of vaccinal strains of Types 1, 2, and 3, while the rest served as a contact group consisting of 30-70 children Fecal specimens were collected from the children in the contact group before the introduction of the virus into the community and thereafter every ten days up to 130 days after vaccination From the inoculated children fecal specimens were taken selectively, at different dates after vaccination, for two months after the beginning of immunization This confirmed the intensive development in the intestinal canal of the virus administered on immunization

Examination of the feces of individual children in the contact group was carried out in two stages First of all, a suspension of each fecal specimen, after it had been centrifuged and combined with antibiotics, was sown on tissue cultures in order to see whether the virus introduced into the community concerned was present or not In cases where it was present, a further quantitative titration was carried out on a corresponding portion of feces kept in the frozen state, in order to establish the titer of the vaccinal virus which had multiplied in the child's stools by the given date

Virological examination of children in the contact group in communities where a vaccinal strain of one of the three types had been administered in one operation, showed that contact infections, which in three or four months observation involved more than 50 per cent of the children examined, spread quite quickly among them

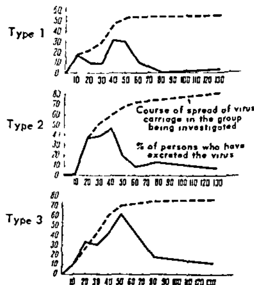


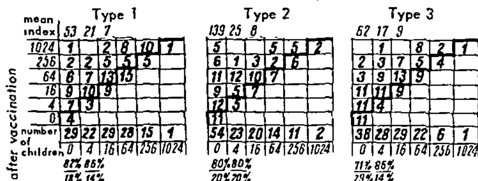
Fig 4 Course of spread of poliomyelitis virus from inoculated children to contact groups

It was this very group of children, the most susceptible to poliomyelitis, which was the main object of our serological examinations

A single administration of 100,000 infective units of vaccine of Types 1, 2, and 3 to groups of children numbering 67-147 persons with no

TABLE 7

GROUPS OF CHILDREN'S ESTABLISHMENTS	AGE OF CHILDREN	NO OF CHILDREN INOCULATED WITH LIVE VACCINE	NO OF CHILDREN IN THE CONTACT GROUPS	TOTAL
Infants' homes	6 months—3 years	633	489	1,122
Pre-school groups in children's homes	4-7 years	845	270	1,115
Total	6 months—7 years	1,478	759	2,237



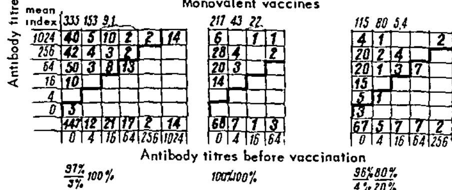


FIG 6 The immunological activity of live poliomyelitis vaccine according to observations on children up to seven years of age in infants' homes and children's homes inoculated with the original Sabin vaccine (Duration of observation one to four months)

The great influence of contact infections by vaccinal strains on the immunological indices among the population is demonstrated by our observations of children's establishments where vaccination had not been carried out, but where the vaccinal virus was imported from vaccinated or contact children transferred to those establishments during 1958 (Table 6)

The data collected underline the particular features of live poliomyelitis vaccination administered per os. The vaccination immunizes not only directly inoculated children but also wide groups of persons in contact with them This is caused by the intensive excretion into the environment of vaccinal strains which have multiplied for one to two months in the intestinal canals of inoculated children In closed children's establishments vaccinal infection involving 50-80 per cent of the contact groups is noted in the three months following immunization.

The intensity and periods of virus multiplication in the intestinal canal of contact children correspond to the indices for directly inoculated children. If 30 to 50 per cent of the children in closed child communities are immunized at one time the highest frequency of contact infection occurs on the twentieth to the fortieth day after immunization.

III. Immunological Changes in Children Inoculated with Live Poliomyelitis Vaccine

The following groups of children up to seven years of age inoculated with the live vaccine or remaining in contact with those who had been inoculated were under our observation in 1957 1958 (Table 7).

Serological examination of children aged six months to three years in infants' homes showed that there were 74 per cent triple negative children among them, in sharp contrast with children in family or crèche groups of the same age (Fig 5).

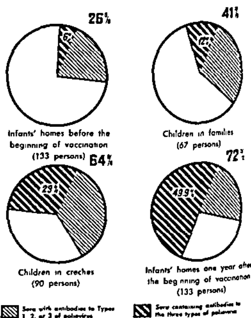


Fig 5 The frequency with which antibodies to poliomyelitis viruses are found in sera from children in organized groups and children not belonging to such groups up to three years of age

Table 6. The Effect of Contact Infections on Immunological Indices in 133 Uninoculated Children in an Infants' Home in Relation to the Repeated Transfer to it During 1957-1958 of Children from Inoculated Communities

Date of Taking of Blood Specimen in a Constant Group of Children (133 Persons)	Immunological Indices in Per Cent		
	Triple Negative	Triple Positive	Antibodies to One or Two Types
Before the beginning of immunization with the live vaccine in the city	74	6	20
One year after the beginning of immunization	28	50	22

By that time the maximum antibody titer, noted two to three months after immunization, had somewhat fallen (to dilution 1:16-1:64) but was nevertheless maintained at medium or high levels in all children inoculated with vaccines of Types 1 or 2. The titers of antibodies to Type 3 were less well maintained, falling in some cases to 0 after 300 or more days.

It is known that similar serological changes are observed also in children who have suffered from the paralytic form of poliomyelitis, in whom. Although the intensity of these changes is less than those shown by the figures given above for monovalent preparations, the immunogenic activity of these combined forms of vaccine is marked by higher indices than that shown after a three-stage injection of the Salk killed vaccine. This applies particularly to the shortened vaccine schedule we used extensively, where the vaccine was administered in two stages (vaccine of Type 1, followed after a month by administration of a divaccine of Types 2 and 3), in this case im-

Types of Antibody	Titers Before Vaccination	% of Positive Response by Indicated Scheme of Immunization			
		Monovalent (1, 3, 2)	Bivalent (1, 2 +3)	Trivalent (1 +2 +3)	Combined (1, 2 +3, 1 +2 +3)
1	0	97	98	82	99
	1:4—1:16	100	88	76	96
	1:64—1:1024	8	21	43	54
2	0	98	84	78	99
	1:4—1:16	100	76	72	86
	1:64—1:1024	19	10	32	49
3	0	94	76	71	96
	1:4—1:16	88	41	48	86
	1:64—1:1024	16	18	52	48

Fig. 9. Homotypic antibody response after 1-3 doses of live attenuated polioviruses by mouth in children 0.5-7 years of age.

after nine to twelve months a gradual reduction is noted in the number of virus-neutralizing antibodies.

A single administration of live vaccine in polyvalent form (100,000 units of each type in a single dose) or double immunization (administration of a Type 1 vaccine followed in one month by administration of a divaccine of Types 2 and 3) provoke satisfactory serological changes in children who had negative indices before immunization (see Fig. 6 at top and Fig. 9).

munity in children who were negative before immunization is maximum against Types 1 and 2 and somewhat lower (up to 80-85 per cent positive antibody shifts) against Type 3 (Table 8).

The high absolute antibody indices in children inoculated with live vaccine and their maintenance for 18 months after immunization at a level not less than that shown by the humoral indices in those who have suffered from poliomyelitis is not only due to the direct action of the live vaccine. Our inoculated groups were in

homologous antibodies before vaccination, caused, after one month, an intensive growth in antibody titer in 97 per cent of children inoculated against Type 1, 100 per cent of those inoculated against Type 2, and 96 per cent of those inoculated against Type 3 (Fig 6, at bottom)

The *mean antibody titer was 335 in those vaccinated* against Type 1, 217 in those vaccinated against Type 2, and 115 in those vaccinated against Type 3

In children who had homologous antibodies before immunization with the live vaccine, serological changes were less marked and less regular than in children of the negative group, but even in these cases there occurred a noticeable strengthening of general humoral immunity

In a constant group of 70 triple negative children aged between one and three years, inoculated at a month's interval with vaccines of Types 1, 3, and 2, we observed the duration of immunological changes for 192 days (Fig 7)

The mean antibody titers by that date were being maintained firmly at levels of 1 960 against Type 1, 1 612 against Type 2, and 1 680 against Type 3

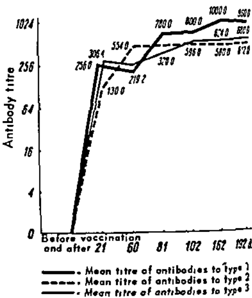


Fig 7 The course of increase in the number of antibodies in children inoculated with monovalent vaccines of Types 1, 2, and 3 (Mean figures for groups of 70 children)

Observations were carried out over a still longer period in the case of small groups of children examined for 18 months (Fig 8).

Fig. 8 Antibody curves in children aged one to three years old inoculated with live vaccine against poliomyelitis of Types 1, 2, and 3.

By that time the maximum antibody titer, noted two to three months after immunization, had somewhat fallen (to dilution 1 16-1:64) but was nevertheless maintained at medium or high levels in all children inoculated with vaccines of Types 1 or 2 The titers of antibodies to Type 3 were less well maintained, falling in some cases to 0 after 300 or more days

It is known that similar serological changes are observed also in children who have suffered from the paralytic form of poliomyelitis, in whom after nine to twelve months a gradual reduction is noted in the number of virus-neutralizing antibodies

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Fig 9 Homotypic antibody response after 1 3 doses of live attenuated polioviruses by mouth in children 0.5-7 years of age

TABLE 8 IMMUNOLOGICAL CHANGES IN THE BLOOD OF CHILDREN AGED ONE TO THREE YEAR INOCULATED AGAINST POLIOMYELITIS WITH A MONOVALENT VACCINE OF TYPE 1 AND A DIVALENT VACCINE OF TYPES 2 AND 3

ANTIBODY TITER	Antibodies in the Blood: to Type 1 (After)						to Type 2 (After)						to Type 3 (After)					
	After vaccination																	
1024	5	—	—	—	—	—	3	—	—	—	—	1	—	—	—	—	—	—
256	2	—	—	—	—	—	7	1	—	—	—	—	1	—	—	1	—	—
64	2	—	—	2	—	—	—	—	—	1	—	—	1	—	1	—	—	—
16	1	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	2	1	—	—	—	—
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Before vaccination																	
NUMBER OF CHILDREN	*10*	—	—	*2*	—	—	*10*	*1*	—	*1*	—	*1*	*10*	*1*	*1*	*1*	—	—
ANTIBODY TITER	0	4	16	64	256	1024	0	4	16	64	256	1024	0	4	16	64	256	1024

constant contact with uninoculated children, among whom contact vaccinal infections gradually spread, which possibly served for the inoculated children as an essential stimulus to the maintenance and continuance of their post-vaccinal immunity

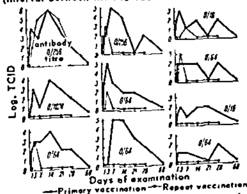


FIG 10 Curves of multiplication of vaccinal strains poliovirus of Type 1 on primary and repeat peroral administration to children aged six months to two years.

The most important feature of the live vaccine is its ability to stimulate the development of local immunity in the intestinal canal. This was shown in the substantial limitation of multiplication of homologous vaccinal strains administered to groups of inoculated children six months after their primary immunization.

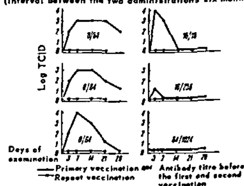


FIG. 11 Curves of multiplication of vaccinal strains of poliovirus Type 3 on primary and repeat peroral administration to children aged six months to two years.

Figures 10 and 11 illustrate typical examples of sharp changes in the indices of multiplication of vaccinal strains of Types 1 and 3, if they are again introduced into the intestinal canal of immunized children

In rarer cases the secondary reinfection of the intestinal canal is accompanied by an inconsiderable multiplication of the vaccinal strains, of a nevertheless temporary and limited character. Only in one case did the repeat administration of Type 1 vaccine reproduce the multiplication indices observed in primary immunization (Fig 10).

The development of local resistance in the intestinal canal in those inoculated with the live vaccine is the most important prerequisite for the gradual cessation of circulation of the wild street strains in inoculated communities The eradication of natural reservoirs of the poliovirus and the consequent liquidation of this infection will proceed with the more speed, the more extensive the inoculations with live vaccine among susceptible groups of children

Conclusions

1 The authors studied the course of multiplication of vaccinal Sabin strains in the intestinal canal of more than 360 children inoculated with monovalent vaccines of Types 1, 2, and 3 The enteral administration of live vaccine in a concentration of 100,000 tissue doses calls forth an active vaccinal process in the intestinal canal of susceptible children, manifested in the intensive multiplication of the administered virus for 30-45 days, reaching a maximum between the seventh and the twentieth day, and the gradual elimination of the virus after 30 60 days The concentration of virus excreted from the intestinal canal fluctuates within wide limits, depending on the level of specific immunity in the inoculated children and other less well studied factors.

2 Identical curves of multiplication of the vaccinal strains and immunological changes in inoculated children were obtained if the dose was reduced to 10,000 tissue infective doses No strict correlations were observed between the intensity of multiplication of the virus in the intestinal canal and the immunological changes in the blood, which attain great intensity in children inoculated with a full dose of vaccine (100,000 infective doses) even when the indices of virus reproduction are only moderate Low or negative reproduction does not stimulate the development of immunity.

3 The vaccinal virus spreads regularly to groups of children in close and long-standing contact with those inoculated with the live vaccine and examined up to six months after the beginning of vaccination The intensity of spread of the virus depends on the age of the children and the sanitational and hygienic conditions under which they live.

4 Immunological changes which were quite regular, intensive and adequately maintained for the 15 months of observation were established in 95 per cent of the groups of vaccinated children susceptible to poliomyelitis The quantitative indices showing a rise in the titer of virus-neutralizing antibodies, and their regularity and length of time for which they are maintained, considerably exceed in their intensity the analogous changes which take place in children vaccinated three times with the Salk killed vaccine Quite marked humoral changes take place also in children with partial immunity before vaccination and also in children in contact with the vaccinated

5 According to the data showing quantitative indices of immunological changes in inoculated children a more convenient type of vaccination for mass campaigns can be used consisting of a two-stage vaccination schedule (a monovaccine of Type 1 followed by a divaccine of Types 2 and 3) as well as the best method three-stage immunization with monovaccines of Types 1, 2, and 3, the intervals between inoculations should be one to one-and-a-half months

6 The administration of live vaccine stimulates the development of local immunity of the intestinal canal and this is manifested by the fact that the multiplication of homologous serotypes of vaccinal strains, if they are administered a second time within the six months of observation, is considerably restricted.

Part 3. Material for the Study of the Harmlessness of the Live *Poliomyelitis* Vaccine *Prepared* from Sabin Strains

A. A. Smorodintsev, E. F. Davidenkova, A. I. Drobyshevskaya, T. E. Klyuchareva, V. I. Ilyenko, O. M. *Chalkina*, K. G. *Vasiliev*, E. V. Glynskaya, V. I. Votiakov, and E. V. Feldman

Department of Virology, Institute of Experimental Medicine of the USSR Academy of Medical Sciences

The first important victory in specific prophylaxis against poliomyelitis was won when the killed Salk vaccine was introduced into practice, which substantially limited the possibility of the virus spreading from the intestinal canal into the central nervous system of infected children by stimulating in 60-70 per cent of inoculated persons a general humoral immunity The killed *vaccine, however, did not eliminate the danger* of paralytic forms and death occurring in 30 per cent of triple vaccinated children and had absolutely no effect on the circulation of the virus in the human community The hopes of long lasting post-vaccinal immunity in those vaccinated with the killed product were not justified, as is shown by the fact that poliomyelitis may develop one to two years after triple immunization with the Salk vaccine and that antibodies regularly disappear six to nine months after completion of immunization in 70 per cent and more of inoculated children

In view of this, it becomes essential to revaccinate with the killed vaccine every two or three years and this makes for serious complications in vaccination practice

All the more importance, therefore, attaches to the swift development of a better and more radical *method of preventing poliomyelitis* by enteral immunization with the live attenuated vaccine, which reproduces the inapparent form of poliomyelitis which prevails under conditions of natural infection *but without the danger of* subsequent lesions to the central nervous system

A valuable initiative was taken in this respect by Sabin, who obtained strains of a low degree of pathogenicity for monkeys but which retained a high degree of immunogenicity for children and adults susceptible to poliomyelitis.

To make it possible to use in practice the live vaccine made from the attenuated Sabin strains it is necessary to carry out extensive observations on human beings in order to study the stability of the inherited properties of the strains under the conditions of natural or artificially caused circulation through the intestinal canal of susceptible persons This task was carried out by A A Smorodintsev, T E Klyuchareva and A I *Drobyshevskaya in 1957-1958.*

It was necessary also to prove the harmlessness of the live vaccine in practice by direct observation of susceptible children, examining for this purpose not only inoculated groups of *children* but children coming into contact with them, in whom the vaccinal virus could undergo lengthy passage and possibly thereby intensify its pathogenicity.

The authors assumed the responsibility for carrying out these tasks in gradually expanding observations on children carried out in the following sequence

1. April 1957 to April 1958 Clinical observations on a group of 150 children aged six months to three years triply vaccinated with a live vaccine of Types 1, 3, and 2, and an equal number of children in contact with them

2 May 1958 to December 1958. Similar clini*cal observations on a group of 822 children* aged six months to three years, and 1,115 children aged four to seven years.

3 January 1959 to May 1959 Study of the harmlessness *of the live vaccine in a community* of children between nine months and fourteen years old, numbering altogether about 12,000, and equal numbers of children in contact groups *(internal contact and external control)*.

4 March 1959 to June 1959 Study of the harmlessness of the live vaccine in mass observations on children vaccinated at ages from six

months to fifteen years, numbering altogether more than 1,800,000

I QUESTIONS OF THE REVERSION OF THE NEUROTROPIC PROPERTIES OF THE ATTENUATED SABIN STRAINS IN LENGTHY PASSAGE THROUGH THE INTESTINAL CANAL OF HEALTHY CHILDREN

The vaccinal strains used in the production of various live vaccines must be harmless for human beings under the chosen method of administration and must be sufficiently stable both during multiplication in the organism of the inoculated person himself and after lengthy natural or artificially caused circulation through the groups of vaccinated persons

The solution of this problem for the live peroral poliomyelitis vaccine depends on the study of the neurotropic properties of the vaccinal strains on intraspinal or intracerebral injection of the live vaccine into Macaca rhesus or Macaca cynomolgus monkeys, which are highly susceptible to the poliovirus The vaccinal strains should possess a low level of neurotropic activity both in their initial state and after prolonged passage through the intestinal canal of susceptible, unvaccinated children

In a number of works devoted to a study of the Koprowski and Sabin vaccinal strains, it was established that their neurotropic activity for Macaca rhesus and Macaca cynomolgus monkeys could intensify in the process of multiplication in the intestinal canal of inoculated persons (Sabin, Dick, Verlinde) The indices of this intensification were particularly high for the Koprowski strains (Dick) According to Sabin's data, his strains, which showed an increase in neurotropic activity for the lower monkeys, remained non pathogenic for chimpanzees

The stability of the hereditary properties of vaccinal strains of the poliomyelitis virus does not necessarily mean that they maintain to the full their initial low indices of neurotropic activity for monkeys after lengthy circulation through the intestinal canal of inoculated persons It is essential that the vaccinal strains should in these conditions continue to show sufficiently large quantitative differences from the street strains of the virus isolated from paralytic poliomyelitis cases, even from those with minimum indices of neurotropic activity

It should be emphasized that the level of neurotropic activity for monkeys is not the only indicator of the harmlessness of vaccinal strains used against poliomyelitis No less important is the degree of affinity of the vaccinal strains for various tissues of the intestinal canal According to Sabin's data his attenuated strains multiply intensively in the epithelial lining of the intestinal tract but have lost that ability to invade the lymphatic system of the intestines, which is a characteristic and constant feature of the pathogenic strains of the poliovirus and which is responsible for their high capacity for generalized spread from the intestinal canal into the circulation and the central nervous system The attenuation of the affinity of vaccinal strains for the lymphatic tissue of the intestinal canal must be considered as a no less important, or even a more important, sign of attenuation than the loss of neurotropic qualities The preservation in vaccinal strains of a certain residue of uneliminated neurotropic activity for monkeys may prove safe for the organism of vaccinated susceptible children for the following main reasons

1 The sensitivity of the central nervous system to the poliovirus is considerably lower in human beings than in monkeys, although the correlations between the neurotropic activity of the poliovirus for monkeys and human beings are insufficiently clear

2 A small amount of residual neurotropic activity for monkeys in the vaccinal strains may not be a reason for considering the live vaccine harmful, if the invasive properties of the attenuated strains have been sharply reduced in the very first stage of their movement towards the central nervous system—from the epithelium to the lymphatic system of the intestinal wall

Our investigations were carried out in infants' homes on healthy children aged two to four years with no antibodies to the vaccinal strains administered For each subsequent passage of the vaccinal strain three to four children were chosen, their blood was examined before vaccination and two months after vaccination, and the progress of multiplication of the strains being passaged was studied after one, three, seven, fourteen days, and at intervals of seven days thereafter

The main criterion for the study of changes in the neurotropic properties of vaccinal strains isolated in monkey kidney monolayer tissue cul

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spinal medulla, which, more often than the clinical data, showed an increase in the neurotropic activity of the passage strains

An analysis of the results obtained from ten consecutive transfers of vaccinal viruses through the intestinal canal of healthy, susceptible children, shows the periodic appearances of strains of Types 1, 2, and 3, with higher indices of neurotropic activity for monkeys than the initial vaccinal strains uneliminated residue of pathogenicity for monkeys, which shows up more clearly in the human intestine than in monkey kidney tissue cultures. The vaccinal strains used for studying the live vaccine are sufficiently stable in their inherited characteristics and only show a limited degree of reversion from their initial neurotropic virulence, at the same time maintaining a low level of pathogenicity for monkeys on intracerebral injection.

TABLE 9 CHANGES IN THE NEUROTROPIC ACTIVITY FOR MACACA RHESUS MONKEYS OF VACCINAL STRAINS OF TYPES 1, 2, AND 3 DURING PROLONGED PASSAGES THROUGH THE INTESTINAL CANALS OF SUSCEPTIBLE CHILDREN

TYPE	METHOD OF INFECTION	INITIAL VIRUS (7 0 LOG 10)	NEUROTROPIC ACTIVITY DURING PASSAGE									
			NO OF PASSAGE									
			1	2	3	4	5	6	7	8	9	10
1	Intracerebral	—	—	—	—	5 5	—	—	—	—	—	—
	Intraspinal	—	5 5	—	6 5	3 5	4 5	6 5	5 5	6 5	—	—
2	Intracerebral	—	—	—	—	5 5	—	—	6 5	—	—	—
	Intraspinal	—	5 5	—	—	5 5	—	6 5	6 5	—	—	—
3	Intracerebral	—	—	5 5	—	5 5	—	—	—	—	—	—
	Intraspinal	—	—	3 5	5 5	3 5	3 5	6 5	—	6 5	—	—

The minimum paralytogenic dose on intracerebral injection was reduced once (to 5 5 log 10) for Type 1 virus, twice (to 5 0 and 6 0) for Type 2 virus, and twice (5 5) for Type 3 virus An increase in neurotropic virulence was observed considerably more often on injection of greater sensitivity 7 times out 10 for Type 1 (up to 3 5 log 10), *4 times out of 10 for Type 2* (5 5 log 10), and 6 times out of 10 for Type 3 (3 5 log 10)

The periodic intensification of the neurotropic activity of vaccinal strains in the intestinal canal of susceptible children was not of a stable and progressive character, the more active viruses regularly returning to their initial non-pathogenic state in subsequent passages This shows the existence in the vaccinal strains of a certain

The negative results of investigation of the neurotropic virulence of virus strains after 9 and 10 passages, even on intraspinal infection of monkeys, are worthy of special attention

These strains are now being split into separate plaques for the isolation of pure lines and for subsequent testing of stability of hereditary characters *in the intestinal canals of healthy children* If they cease to split off more virulent variants and preserve a high capacity for multiplication in the intestinal canal and most immunogenic properties, it will be possible to use them as producers of maximum pathogenicity for making live vaccine

Similar results were obtained by the testing on monkeys of the neurotropic virulence of 14 vaccinal strains, isolated from contact groups of

tures after each successive passage through the intestinal canal of healthy children was the titration of the virus-containing liquid on Macaca rhesus monkeys by intracerebral or intraspinal injection in concentration of virus 6.5, 5.5, 4.5 $\log_{10}$ (for intraspinal also 3.5 $\log_{10}$ was used). Two to three monkeys were chosen for each dilution and only fresh animals which had not undergone any experiments, were used.

The monkeys were infected under Amytal sodium or ether narcosis, or without narcosis, by the injection of 0.2 ml. of virus containing fluid into the lumbar enlargement of the spinal medulla (the fourth intervertebral space counting up from the iliac crest). The virus was injected through a No. 20 needle 1.5 cc long somewhat to the side of the center line. Proof that injection had been successfully made into the grey matter of the anterior horns of the lumbar enlargement was the occurrence of fibrillary contractions in the various groups of muscles of the hind extremities at the moment of insertion of the needle and injection of the liquid, and also the data obtained from selective examination of that zone of the spinal medulla of monkeys sacrificed 21 days after injection (the occurrence of a scar at one or more of the 20 levels of the brain examined). All of the injections were done by same person (T. E. Klyuchareva). The monkeys were examined 1 and 24 hours after injection. The single cases found of quickly developing traumatic paralysis after intraspinal injection were excluded from the experiments. The animals, fixed to a long chain, were observed daily for 21 days, by examining their movements through the wire-netting walls and ceiling of the cage in a watch for paresis and paralysis. These developed from the fourth or fifth day and continued to the 14th-21st day in the form of sluggish paresis and paralysis of the extremities of varying degrees of severity. Sometimes they were preceded by a small rise in temperature which was, however, irregular in character. The material used for infection of the monkeys was virus-containing liquid taken from kidney tissue monolayer cultures infected with the feces of children vaccinated with a passage virus of Type 1, 2, or 3, the specimen being taken between the 14th and 28th day after vaccination. After the biological activity of the isolated strain had been titrated in tissue cultures and it had been iden*tified by means of type-specific serum*, its neurotropic activity for monkeys was studied.

After completion of the tests on monkeys and of all the tests for the live vaccine, including a sterility check and a check for absence of tuberculosis bacillus and of virus B, the isolated virus was administered in milk during the two subsequent days to a new group of children in a dose of 100,000-200,000 TID$_{50}$. Three to four months were spent on each passage.

Beginning on the second day after vaccination with the passage strain, the course of multiplication of the passage virus in the intestinal canal of inoculated children was studied. Figure 12 shows the curves of multiplication of the passage strains of virus of Types 1, 2, and 3.

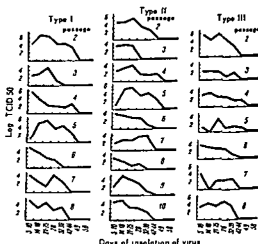


FIG. 12. The course of multiplication of passage strains of poliovirus of Types 1, 2, and 3 in the intestinal canal of susceptible children.

During all eight consecutive passages the viruses maintained their initial activity for the intestinal canal and provoked intensive development of antibodies in the vaccinated children. This shows that the attenuated strains have a reliable immunogenic potency not only in the first control groups but also after prolonged circulation through the intestinal canal of vaccinated persons.

Table 9 shows the results of tests of the intracerebral and intraspinal activity of the passage strains, expressed by a log 10 index causing specific paresis or paralyses in infected monkeys. To evaluate the results, only clinical data were used, *and not histopathological changes in the*

TABLE 10 INFORMATION CONCERNING SUSPECTED OR CONFIRMED CASES OF POLIOMYELITIS AMONG VACCINATED AND UNVACCINATED PERSONS IN LENINGRAD

Surname and Initials	Age	Date of Vaccination of the Patient and Types of Vaccine	Date of Vaccination in the Community Concerned	Length of Time After Vaccination Before Development of the First Symptoms	Markedness and Duration of Paralysis	Virological Data	Serological Data	Bacteriological Data
Fedotov, S	4	— (contact)	21 May Type 1	40 days	Meningeal syndrome, acute cytosis	—	—	Meningococcus was isolated from the cerebrospinal fluid
Stasi, D	2	16 October Type 1		90 days	Meningitis, acute pleocytosis	—	—	Pneumococcus was isolated from the cerebrospinal fluid
Filimonov, S	1 year, 4 months	30 April Type 3		8 months	Paresis of the left facial nerve	Poliomyelitis virus of Type 1	—	—

children, 3-4 months after the introduction of the virus into the community concerned. It turned out that when there were repeated natural passages through the intestinal canal the indices of neurotropic activity of the vaccinal strains changed no less considerably and regularly than during artificial transfers from one group to another.

The inability of the human intestine to select and maintain a progressive accumulation of neurotropic and highly invasive variants of the poliovirus is also proved by observations on the neurotropic activity of variants of the virus circulating naturally among healthy people During its thousands of years of symbiosis in the intestinal canal of the human being, the poliomyelitis virus has remained one of the least pathogenic disease agents, causing disease in one child out of 200 (Type 1) or even one child out of 500-1,000 (Types 2 and 3)

This feature was confirmed also by our observations on the reversion of vaccinal strains

II The Harmlessness of Live Vaccine Made From Sabin Attenuated Strains in Observations on Children

The first stage in the tests of harmlessness of the live polio vaccine was the clincial examination in Leningrad of 2,335 children from infants' homes and from the pre-school groups in children's homes, who had been vaccinated or in contact with vaccinated children in 1957-1958

The observations were carried out by two neuropathologists, Professors E F Davidenkova and E. A Savelyeva-Vasilyeva, and by an expert in infectious diseases, Professor V V. Kosmachevski, and his assistants Of the 1,122 children aged six months to three years under observation 74 per cent were triple negative. Of the 1,115 children aged four to seven years, more than 50 per cent were negative to one of the three types of virus

The physicians attached to these communities of children selected, under the guidance of our consultants, somatically healthy children, with constantly normal temperature, who had not been in contact with other infections and had not been injected with other vaccines for a month or more beforehand Children with irregular intestinal function and those convalescing after gastric and other diseases were excluded The children's temperatures were taken regularly morning and evening and their general condition, pharynx and intestines were kept under observation

In some of the vaccinated children the general blood picture, the erythrocyte sedimentation rate, and their urine and feces were checked before and after vaccination Special attention was paid to the condition of the nervous system in each vaccinated child or child in contact with those vaccinated, taking into consideration not only clearly-marked symptoms of poliomyelitis, but the slightest signs of the inapparent or abortive forms Children with suspect nervous symptoms were sent to the hospital for more detailed observation in the special poliomyelitis department of the Nerve Clinic of the Leningrad Institute of Medical Pediatry.

Each child from the vaccinated or contact group was under the clinical observation of specialists for between one and two years

Some of the infants' and children's homes in Leningrad served as an "external control" into which the virus was not imported but where the children were kept under just as careful observation as in the communities where immunization had taken place

Of the total number of 1,507 vaccinated children and 828 children of the same age-groups in contact with them, not one showed any local or general reactions There were no findings of nausea, vomiting, or intestinal dysfunction The blood and urine analyses and erythrocyte sedimentation rates showed no changes compared with the figures before vaccination

In not a single case among the vaccinated or contact children did poliomyelitis develop in the six months immediately after vaccination There were no changes in motor functions, gait, tendon reflexes or the tone of individual groups of muscles, or even the slightest meningeal or radicular symptoms, tenderness in groups of muscles or changes in the vegetative nervous system

Table 10 shows cases of illness with nervous symptoms observed among vaccinated or contact children during the two-year period of observation.

The two cases of meningococcal and pneumococcal meningitis are obviously diseases of another kind superimposed on vaccination after it has been done. The case of the baby, S Fili-

TABLE 11 ANALYSIS OF THE REACTION-CAUSING PROPERTIES OF LIVE POLIOMYELITIS VACCINE ACCORDING TO DATA CONCERNING VACCINATIONS IN THE SCHOOLS IN THE LENINSK RAYON OF THE CITY OF RIGA IN THE LATVIAN SSR

(K. G VASILIEV, E V. GLYNSKAYA)

The figures are for the sickness rates in the groups mentioned per 10,000 persons (of the total number of illnesses registered during four months of observation).

Recorded Diseases	Rate of Sickness Among the Groups Mentioned		
	Live Vaccine Schoolchildren 8311 Children of Pre-school Age 1346	Internal Control Schoolchildren 4188 Children of Pre-school Age 768	External Control Schoolchildren 3300 Children of Pre-school Age 1062
I Poliomyelitis and similar diseases	—	—	9 3
(aseptic meningitis, meningeal phenomena, other diseases with nervous symptoms	—	—	—
II Acute infectious diseases (influenza, acute catarrhs of the upper respiratory tract, scarlet fever, diphtheria, mumps, chicken-pox, dysentery, infective hepatitis, etc)	3,697 6	3,662 3	3,506 0
	2,526 0	2,556 4	2,821 8
III Diseases of the respiratory organs and ear, nose and throat (pneumonia, bronchitis, rhinopharyngitis, sore throats, tonsilitis)	474 5	496 0	472 7
	1,315 0	1,119 8	1,468 1
IV Diseases of the digestive organs (gastritis, gastroenteritis, enteritis, colitis, appendicitis, etc)	21 5	23 4	18 1
	74 3	78 4	75 3
V Other diseases (pyelitis, nephritis, bronchial asthma and lymphadenitis)	10 3	22 3	21 1
	29 7	26 0	28 2
VI Injuries	2 4	2 5	12 1
	—	—	—

age, has been carried out, there has been no increase in the past months in the incidence of poliomyelitis compared with similar months of 1957 and 1958 Statistical calculations of the expected number of poliomyelitis cases showed in April, and particularly in May, not an increase but a decrease in the cases expected by between two and four fold

monov (paresis of the facial nerve with isolation of poliovirus of Type 1), does not indicate that the vaccine is harmful, since the disease developed eight months after a single immunization with a vaccine of Type 3, nor does it detract from the effectiveness of the live vaccine, since this child had been transferred a fortnight after immunization to another establishment and had not completed the cycle of inoculations.

Among the inoculated and contact children in one infants' home, seven months after the beginning of immunization a small outbreak of illness with nervous symptoms occurred Examination of the feces of the sick children led to the isolation of seven strains of the same type of Coxsackie group A, pathogenic for newborn mice, and an increase in the number of antibodies in the blood of the sick persons to the isolated strains

During the one or two years' observation, outbreaks of various infectious diseases occurred among the inoculated and contact groups (influenza, chickenpox, scarlet fever, etc) which followed a completely typical course and showed no tendency to greater severity among vaccinated children This applies also to the mass outbreaks of influenza in September 1957 and in February 1959, which were not marked by increased severity among vaccinated children

The data obtained from prolonged clinical observation of these children make it possible to conclude that the live vaccine made from the Sabin attenuated strains has been completely harmless and has caused no reactions among 1507 vaccinated children of pre-school age in contact with 828 healthy children of the same age.

The second stage in testing the harmlessness of the live vaccine was carried out by specialists in the City of Riga (K. G Vasiliev, E V. Glynskaya, etc) on 9,657 children vaccinated in two stages with a live vaccine of Type 1, followed by a divaccine of Types 2 and 3, with a control group of 4,956 children of the same age in the establishments where vaccination had been carried out, these serving as an internal control, and 4,362 children serving as an external control Serological examination of selected groups of children of various ages before vaccination showed that 42 per cent of them were negative to Type 1 and about 40 per cent to Types 2 and 3

Table 11 shows all illnesses recorded in the four months following the beginning of vaccination among vaccinated and unvaccinated control children (internal and external controls), calculated on a basis of 10,000 persons.

Analysis of the data obtained shows the complete absence of poliomyelitis cases among the vaccinated and internal control group for the four months of observation following administration of the Type 1 vaccine and the Types 2 and 3 divaccine. All the other diseases of a different kind recorded during this period of observation and belonging to the categories of acute infection, respiratory tract diseases, digestive ailments, etc , showed identical distribution among the vaccinated children and the internal and external control groups.

The third stage in testing the harmlessness of the live vaccine was begun in April 1959 in four different regions of the USSR and covered 1,423,645 children aged six months to 15 years, who were given vaccine of Type 1 followed after a month by a divaccine of Types 2 and 3 On 1 June 1959 immunization covered the following groups

Region	*Total vaccinated in the two stages*
1	46,045
2	411,000
3	544,600
4	422,000
Total	1,423,645

Preliminary serological examinations of about 1,000 sera from children of various age-groups vaccinated in these regions show that nearly 30 per cent of the total number of vaccinated children had had negative antibody findings before immunization in respect to Types 1, 2, and 3. This gives a total of more than 500,000 persons on whom the administered live vaccine could show its pathogenic properties

The results of medical and epidemiological observations of reactions among those vaccinated during the two months following immunization have established the following facts:

(1) The live vaccine in practice causes absolutely no reactions

(2) In all the regions where mass immunization, covering between 60 and 90 per cent of the total number of children up to 15 years of

CHAIRMAN GEAR. Professor Smorodintsev's paper is now open for discussion

DR STUART HARRIS The figures presented by Dr. Smorodintsev must carry considerable weight with anyone who is planning the use of live poliovirus vaccine. It would be interesting to know what proportion of the children living in any one area were actually given live vaccine during these trials

The reason I ask is simply this that obviously the greatest likelihood of multiplication of virus exists in areas where a very small number of children are given vaccine, and a large number are left unvaccinated It would therefore be interesting to know what the proportion of children was in the various areas

DR SMORODINTSEV We vaccinated in different republics from 60 to 85 per cent of the whole child population below 15 years of age

DR STUART-HARRIS I have another question This is a much smaller question and arises from the statement that Dr Smorodintsev made about the increase in neurotropic virulence, which was stated to happen more often "with greater sensitivity" Does the use of that phrase refer to the intraspinal inoculations? If so, could Dr Smorodintsev tell us exactly what is his criterion for defining an increase of neurotropic virulence?

DR SMORODINTSEV: The original Sabin strains are practically of very low pathogenic activity for Macaca rhesus monkeys—even in the amount of 7 0 logarithms of 10, by introduction intraspinally. We do not observe practically any evidence of paralytic symptoms in inoculated monkeys, but we can find histopathological changes in the same animals

If you compare these data with strains isolated from the intestinal tract after some passages through susceptible children, you may find evidence that increase occurs, but the quantitative level of this increase is not more than three logarithms of 10 as with the maximum shown by the virus After one or two passages we obtained again virus with original very small pathogenic activity, as it was very typical for the original strains

DR HAMMON I wonder if Dr Smorodintsev would be kind enough to go back to the table on the pathogenicity of the viruses after numerous human passages, 1 to 10 I was unable to grasp the meaning of this table as it was on the screen and I think there are probably some others that need some help on this

DR SMORODINTSEV I shall refer to this table here

CHANGES OCCURRING IN THE NEUROTROPIC ACTIVITY OF VACCINE VIRUSES AFTER ITS PROLONGED AND REPEATED PASSAGES IN THE ALIMENTARY TRACT OF TRIPLE NEGATIVE CHILDREN

TYPE	METHOD OF MAC RHESUS INFECTION	ORIGINAL ATTENUATED VIRUS (7 0 LOG 10)	PARALYTOGENIC DOSE OF VIRUS (IN LOG 10) AFTER INDICATED NUMBER OF PASSAGES									
			1	2	3	4	5	6	7	8	9	10
I	i/cer	—	—	—	—	5 5	—	—	—	—	—	—
	i/spin	—	5 5	—	6 0	3 5	4 5	6 5	5 5	6 5	—	—
II	i/cer	—	—	—	—	5 0	—	—	6 0	—	—	—
	i/spin	—	5 5	—	—	5 0	—	6 5	6 0	—	—	—
III	i/cer	—	—	5 0	—	5 5	—	—	—	—	—	—
	i/spin	—	—	3 0	5 5	3 5	3 5	6 5	—	6 0	—	—

(3) Of the number of single cases of poliomyelitis recorded in the regions where vaccination was carried out in April-May 1959, there is not a single case which could possibly be connected with vaccination

All the corresponding documents will be presented in more detailed form later

CONCLUSION

The experimental, clinical, and epidemiological observations quoted show the harmlessness of the live vaccine made from attenuated Sabin strains and the possibility of using it on a wide scale for the mass immunization of children and adolescents, in order further to reduce the number of paralytic cases and to eradicate poliomyelitis as a mass disease

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he has done it—in which he takes five children, puts them in contact with five triple negatives, studies the fecal virus, takes these children and puts them in contact with five other children

That would more truly represent what happens in nature, and give us more precise information on changes which occur with these viruses

The other question I would like to ask is this How many years does Professor Smorodintsev estimate it would take before we have precise evidence of the efficacy of the vaccination in the republics where it has been studied?

Dr. Smorodintsev: If the vaccine proves to be highly effective, as we hope, it may be studied in a very short time, perhaps next summer or autumn season during this year, provided it is able to suppress markedly the illness among vaccinated people; but if this vaccine is not as effective as may be supposed in connection with immunological data, it of course may take much more time to estimate its efficiency in condition with very low morbidity rates.

But we need, of course, very large groups for observation, which are in different immunological conditions Therefore, in our country, in six different republics, and Dr Chumakov will report to you very soon about his work, we have sufficient material now—about three and a half million vaccinated children—for estimation of the epidemiological efficiency during the next season and perhaps during the next years.

As regards monkey experiments, I will be very happy if Dr. Dick himself will pay more attention to these questions, because until now I have conducted sufficient passages, and I think that it is not necessary to prolong these passages further, even in the very nature of conditions, as he recommends now

We are now occupied with our immunological and epidemiological work, and I consider that it is a most important task, to decide the safety and efficiency of a new preparation

Dr Paul I wish to ask Professor Smorodintsev if he would elaborate a little more on his concept of "local immunity" This seems to me to be an extremely important subject, only because it introduces a new concept of resistance, which may eventually rank with antibody production as a measure of immunity to live virus infection

The concept of local immunity is more or less an unexplored field It brings out new principles of resistance to this disease, over and above that which the Salk vaccine produces If it is as important as the work of Professor Smorodintsev and others seems to indicate, there ought to be methods for measuring it

Dr. Smorodintsev: The mechanism of this local resistance is quite obscure I know only that this local resistance is type specific and perhaps is connected with antibody, with local antibody production which should be measured not in the feces directly, but in material which should be very concentrated and collected directly from the mucosa of immune children

We estimate this resistance through direct introduction of vaccine strains after different periods of immunization. Control is, of course, very important in further studies at the time when this resistance is still evident The period of six months that we used is not sufficient, and now we prolong this work with children who were vaccinated one and two years before to study the persistence of this resistance to this longer time. I think also that now it is extremely important to keep attention on the distribution of polio wild viruses in areas where vaccination is made, because the disappearance of poliovirus may be the most important indication of the effect of this method

We started in Leningrad with observations on selected and sufficiently large groups of children who were immunized by live vaccine and by Salk vaccine, and this investigation, made during last year, showed a very distinct, a very sharp difference in carr[illegible]

[illegible]

[illegible]

living in very open conditions in our city of Leningrad, when they were immunized with live vaccine

If they were inoculated with killed vaccine, we find usually a pretty high percentage of poliovirus carrier stage It is also very important for estimation of the role which local resistance plays in this problem.

The minus means negative clinical symptoms in monkeys by the introduction of original tissue culture in concentration 6.5 log 10, for original strains as also for passaged strains. But if we obtained clinical signs of paralysis by intracerebral or intraspinal injection we have calculated in these figures the minimal quantity of virus that was active by titration. So we have estimated the minimum quantitative level of pathogenic activity which was produced in monkeys by the virus introduction indicated—after a various number of human passages.

DR. MELNICK: I would like to add a comment on this table. At the bottom of this table, for example, where the Type 3 data are shown, a negative means greater than 7 logs of virus failed to paralyze. Thus after the second passage there has been an increase of 4 logs in neurovirulent titer for monkeys. However, if Professor Smorodintsev had used a more sensitive method, namely a fine gauge needle, he might have found a much greater amount of neurotropic activity. We were able to find that 4 logs of vaccine virus of the type used by Professor Smorodintsev contained one paralytogenic dose for monkeys. Thus a further increase of 4 logs in neurotropic activity on passage, as reported by Professor Smorodintsev, would mean that one virus particle (of the passage line) as measured in tissue culture, would be sufficient to paralyze a monkey if inoculated by the most sensitive method.

While I have the floor, I would also like to ask Professor Smorodintsev if he would refer to the paragraph of his paper in which he says: "Similar results were obtained by the testing on monkeys of the neurotropic virulence of 14 vaccinal strains, isolated from contact groups of children, 3 to 4 months after the introduction of the virus into the community concerned. It turned out that when there were repeated natural passages through the intestinal canal the indices of neurotropic activity of the vaccinal strains changed no less considerably and regularly than during artificial transfers from one group to another."

I would like to ask whether he would be good enough to let us have the experimental evidence on which this is based.

DR. SMORODINTSEV: I want to stress that during the prolonged passages in monkeys the results we have obtained by participation of one and the same doctor who used this same quantitative technique during this whole time, about two and a half or three years, did not show any progressive increase of original neurotropic activity which we occasionally observed for different types of virus during the prolonged passages.

I do not know of any evident correlation between these data and actual pathogenicity of these strains for human beings, but I think that the time now is coming when we can much more precisely estimate the actual pathogenic activity of the vaccinal strains under certain conditions, which I reported to you in field trials on more than 1,500,000 children.

I think these data are much more important than some increase of original neurovirulence for two or three logarithms for monkeys.

We should keep in mind that not only neurotropic activity but invasive capacity of the strain through the intestinal tract is perhaps much more important, and as Dr. Sabin has shown, this capacity to invade the tissue of the intestinal tract is markedly reduced in attenuated strains. Therefore, a small residue of neurotropic activity which we practically have, may be of no practical significance, and our experience with large groups of human beings vaccinated in most natural conditions, I think has shown that we have vaccine strains not only good from an immunological standpoint but also really safe.

Between these 14 strains which we have isolated during our contact experiments, we also found single strains which produced in monkeys, by intraspinal inoculation in the amount of 3.5 to 4.0 logarithms, paralytic or paralysis signs.

But this level was not diminished further. These are the results which I think are the most important for the vaccinal strains used in our work.

DR. DICK: This experiment, of course, is a very artificial situation in which you take fecal virus and grow it in tissue culture, and then you give tissue culture material to the next group of individuals. It would seem to me that much more information could be obtained if a careful contact experiment was done in series. I wonder if Professor Smorodintsev has contemplated doing this, an experiment perhaps—we haven't got the details of it here. Dr. Sabin says

he has done it—in which he takes five children, puts them in contact with five triple negatives, studies the fecal virus, takes these children and puts them in contact with five other children

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We started in Leningrad with observations on selected and sufficiently large groups of children who were immunized by live vaccine and by Salk vaccine, and this investigation, made during last year, showed a very distinct, a very sharp difference in carrier stage of polioviruses in these two groups Practically, polioviruses completely disappeared from the child population, which was living in very open conditions in our city of Leningrad, when they were immunized with live vaccine

If they were inoculated with killed vaccine, we find usually a pretty high percentage of poliovirus carrier stage It is also very important for estimation of the role which local resistance plays in this problem.

DR FOX I may be repeating something Professor Smorodintsev already stated, but it seems to me that one of the most important features of this whole study, in terms of the large-scale application of the virus, was the prevaccination immunologic status of the population to which the vaccine was applied His figures provide one with some reasonable estimates, I believe, of very substantial numbers of truly susceptible individuals well up into advanced age groups And this is, from the standpoint of people interested in the use of live virus here in this country, of very substantial importance

I have been concerned in the past about the problem of translating to the United States the experience acquired in more underdeveloped parts of the world, where the immunity pattern is rather different and where the great proportion of the truly non-immune members of the population are in the very young age groups

It seems to me that these Russian data provide experience in a population which immunologically is quite comparable to many parts of this country and they give me much more confidence in going ahead with the use of live virus under these circumstances, especially when one recalls that the age of the host is also a very important factor in determining whether infection is safe or not

DR KOPROWSKI I would like to comment once more on the question raised by Dr Paul in relation to the problem of local resistance to poliomyelitis virus We do not know the mechanism of local resistance but we can perhaps formulate a working hypothesis that the poliovirus multiplying in the intestinal tract of susceptible species causes the formation of anti-viral substances in the regional lymphoid tissue, such as Peyer's patches and the mesenteric lymph nodes

When an individual is re-exposed to a virus with which he has been previously infected, the regional lymph nodes are stimulated to act against the virus either by direct contact of cells with the virus particles or through liberation of an antibody similar to the one observed in serum This inactivates the virus in the intestinal lumen, without the apparent participation of the serum antibody.

This hypothesis can be confirmed if the presence or absence of polio antibodies is studied in the lymph and lymphocytes obtained from the thoracic duct of monkeys fed large doses of poliovirus As we all know, thoracic duct lymph drains from Peyer's patches and mesenteric lymph nodes. The present state of our knowledge makes it impossible to answer Dr Paul's question in exact terms. Our knowledge of antiviral antibody production in relation to local resistance is quite meager and it would be impossible at this point to define its exact mechanism

Several years ago we published the results of our study of six serial passages in man of a Type 1 attenuated strain of poliomyelitis virus Data presented by Dr. Smorodintsev have essentially confirmed our results We failed to detect marked changes in neurovirulence for monkeys following six consecutive passages through the human intestinal tract in Type 1 non immune children More recently, the strain resulting from these passages has undergone three more passages in man, this time in family contact infections Thus, in total, we had under our observation nine passages through the human intestinal tract. There was no indication whatever that the virus representing the highest passage through the human gut was more virulent for monkeys than the original vaccine

I believe that these two trials are sufficiently convincing to make unnecessary the designing of other trials of a similar nature, which would involve inoculation of hundreds of monkeys and which I do not believe would enrich our knowledge in this field

DR BENYESH-MELNICK. Dr Smorodintsev implied that the strains excreted by vaccinated children had lost their invasiveness for the lymphatic system of the intestinal tract. I would like to ask Dr. Smorodintsev if he would be kind enough to give us some data on how invasiveness was tested, and to what degree these strains differ from the vaccine strains in their capacity to penetrate the lymphatic system of the intestinal wall

DR SMORODINTSEV: I think it may be fortunate to keep in mind the disease problem and estimate that difference of vaccine strains, not only in connection with neurotropic activity, but as regards its existence in the tissues in the intestinal tract

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It is obvious that it was intended to provide information to the best of the ability to which it could be carried out, and this was supplemented by his observations on the natural transmissions which were occurring, and which he has reported before and refers to in the statement here

My own interpretation of the monkey tests is that they do not lend themselves to quantitative estimations I think that what they show is what he concluded, that there is no progressive increase in neurovirulence on continued passage in the human intestinal tract, just as there is no continued and progressive increase in neurovirulence in continued multiplication in the individual

Also, they show that whatever increases may occur, there has not been a single instance, either in his studies or in anyone else's in which complete return to neurovirulence has been observed

Obviously, the final answer is the answer that is obtained in the field And that is why the final answer could be gotten only by the field trials that he has described and others have carried out

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Now, in nature there are all kinds of polioviruses in different stages of the spectrum and there is an instance where over a period of forty years, natural passages have continued and give us at least some indication of what can occur under such natural conditions

Now I want to address myself to one more question the question of effectiveness Obviously a live virus vaccine cannot be measured for effectiveness by any of the conventional epidemiological methods where you apply the incidence in unvaccinated people, and compare it with that in the vaccinated, because the vaccine spreads to the unvaccinated and immunizes a portion of the unvaccinated population, furthermore, by virtue of this local resistance in the intestinal tract in a large proportion of the individuals, it interferes with the spread of virus that is introduced and so would also affect not only the vaccinated ones but also the unvaccinated.

Therefore, we are really faced with an important question What are the new epidemiological tools that must be thought of, that must be used, in evaluating such a vaccine which is quite different from any of the others used before?

One thing that the experience of the past years with the Salk vaccine and the gamma globulin studies have provided is a reasonable assumption *that insofar as antibody production has been* measured in such a population and insofar as there has been an antibody response, those with antibody may be assumed to be protected against paralysis, certainly in the same way as children who developed antibody after receiving Salk vaccine are protected against paralysis

The next question is How are you going to tell over the years that you have really influenced the situation for a very long time? We all know that a community may go for a large number of years without a high incidence of paralytic poliomyelitis After the 1916 epidemic in New York City, 15 years elapsed before the 1931 epidemic with very low incidences over a long period of time

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I have been concerned in the past about the problem of translating to the United States the experience acquired in more underdeveloped parts of the world, where the immunity pattern is rather different and where the great proportion of the truly non-immune members of the population are in the very young age groups

It seems to me that these Russian data provide *experience in a population which immunologically* is quite comparable to many parts of this country and they give me much more confidence in going ahead with the use of live virus under these circumstances, especially when one recalls that the age of the host is also a very important factor in determining whether infection is safe or not

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When an individual is re-exposed to a virus with which he has been previously infected the regional lymph nodes are stimulated to act against the virus either by direct contact of cells with the virus particles *or through liberation of* an antibody similar to the one observed in serum This inactivates the virus in the intestinal lumen, without the apparent participation of the serum antibody

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Several years ago we published the results of our study of six serial passages in man of a Type I attenuated strain of poliomyelitis virus *Data presented by Dr. Smorodintsev* have essentially confirmed our results. We failed to detect marked changes in neurovirulence for monkeys following six consecutive passages through the human intestinal tract in Type 1 non-immune children More recently, the strain resulting from these passages has undergone three more passages in man, this time in family contact infections Thus, in total, we had under our observation nine passages through the human intestinal tract There was no indication whatever that the virus representing the highest passage through the human gut was more virulent for monkeys than the original vaccine

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tion, not only over a period of a few months, but over a period of years

The final answer must still be obtained in the end, but the question is not what is the effectiveness, because certainly we can expect effectiveness on the basis of antibody production, if nothing else. We must ask. What is the degree of effectiveness, the extent, and what will happen subsequently? Will the amount of intestinal resistance that has been produced prevent invasion or reintroduction of epidemic strains of virus from other areas?

Those are the questions for the future, and I am sure that epidemiologists who are going to apply themselves to thinking about how to tackle this, may come up with perhaps even better ones; but I hope they will give their thought to new ways of attacking this, because the old ways of testing previously known vaccines, I am afraid, do not apply.

CHAIRMAN GEAR: Thank you, Dr Sabin Professor Smorodintsev, would you want to comment on anything that was said?

DR SMORODINTSEV. I agree completely

TOPIC V. FIELD TRIALS

1. A SMALL-SCALE TRIAL OF TYPE 3 ATTENUATED LIVING POLIOVIRUS VACCINE

PROFESSOR C. H. STUART-HARRIS

University of Sheffield
Sheffield, England

PROF. STUART-HARRIS: This trial has been described in full in the accompanying paper which was published in the *British Medical Journal* in November 1958.* For the purpose of this audience, however, I shall try to give a shortened account which I have made available to our translators, as follows.

In any small scale trial of a living vaccine which seeks to determine clinical reactions, excretion of virus and possible spread of such virus, it is advantageous to be able to keep the inoculated subjects under close supervision and to exclude so far as possible extraneous or wild viruses. We chose as subjects for this trial a group of children aged 6 to 15 years who were patients undergoing treatment for various orthopedic conditions at a long stay hospital. Thereby we hoped to overcome the various difficulties surrounding the control of our subjects and furthermore had a long period of surveillance during which a watch was kept for wild viruses in the stools before the vaccine virus was fed and during which two doses of British-type of Salk vaccine were administered intramuscularly. Type 3 vaccine was chosen deliberately because this type had not been isolated in Sheffield in the several months before the trial. The virus was fed in two successive lots at an interval of 21 days, seven boys received vaccine on the first occasion and eleven, including all those who did not receive vaccine before, on the second occasion. Dr. Sabin's attenuated Leon Type 3 virus was fed in hard gelatin capsules containing polyethylene glycol and dummy capsules containing only culture fluid were fed to seven boys who acted as control contacts during the first stage of the investigation. The dose of virus was 4.8 logs of TCD_{50} of virus in 0.2 ml. volume.

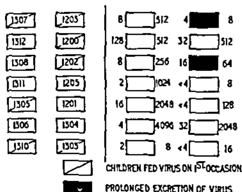


FIG. 1. Neutralization titers, pre-Salk and pre-feeding antibodies (Type 3).

Figure 1 shows the ward in diagrammatic fashion. On the left the various patients are shown by number and the diagonal line shows those who received vaccine virus on the first occasion. To the right the neutralizing antibodies against Type 3 virus before administration of Salk vaccine are shown on the left-hand side of the rectangles. Figures to the right of the rectangles are the antibodies after Salk vaccine and just before the vaccine virus was fed. The 2 black rectangles

* Clarke, S. K. R., Goffe, A. P., Stuart-Harris, C. H., and Herzog, E. G.: Brit. M. J., 2: 1188, 1958.

indicate the 2 boys, 1203 and 1202, who became infected with virus and who excreted it for some days We were surprised that the other five boys receiving virus failed to become infected although one had shown no detectable antibody prior to Salk vaccine administration (1303) and one other (1310) had only a low level of Type 3 antibody at the same time

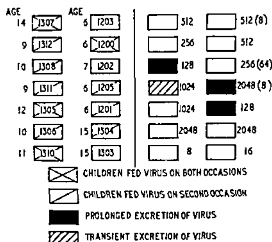


Fig 2 Excretion of virus and post feeding antibodies (Type 3)

Figure 2 shows the second phase of the experiment after eleven boys had received vaccine virus, seven for the first time and four for the second time None of the latter became infected but three of the others did become infected and excreted virus for prolonged periods (1308, 1205, and 1201) A fourth boy (1311) excreted only a trace of virus, probably that remaining from the virus which was fed on the day after vaccine was administered Two of the boys infected in this second phase of the trial had shown no detectable antibody in their serum before Salk vaccine was given and a third had a titer of 8

The number of subjects in this trial was too small for deductions to be made on the relationship between antibody levels and ability to become infected. Table 1 shows the ages of the boys, the antibody response observed and the duration and maximum titer of excreted virus per gram of feces. It was a fact that 2 of the 3 boys who had no detectable antibody prior to Salk vaccine became infected but the other 3 had all shown some antibody presumably due to some previous natural infection before Salk vaccine was given This table shows that 3 of the successfully infected boys were 6 years old and one was 7 and these were mostly the youngest subjects in the trial. An antibody response to Type 3 virus occurred in only 3 out of the 5 boys who were infected and neither antibody response nor excretion of virus occurred in any of the control subjects fed dummy capsules during the first phase of the trial We did not observe any spread of virus to the nurses looking after the patients but these attendants wore rubber gloves during handling of bed-pans and it was a fact that all of the boys who excreted virus were confined to bed on plaster or other frames The duration of excretion of virus in each of the five boys was from 9 to 30 days and the maximum titer per gram of stool was from 3 to 5 logs TCD_{50} for the various subjects

Virulence tests on the vaccine and on the excreted viruses were carried out by Dr Goffe of the Wellcome Research Foundation over 160 miles away In my own laboratory the fecal specimens were all examined by inoculation into tissue cultures of monkey kidney In each case we sent

Table 1 Neutralizing Antibodies and Virus Excretion in Six Children

Number of Child	Age	Antibody Response	Virus Excretion (Days)	Maximum Titer (Day) (TCD_{50} Log_{10})
1202	6	64/256	14	5 2 (6th, 7th)
1203	6	8/512	13	5 0 (5th)
1201	7	128/128	25	3 0 (21st)
1205	6	8/2048	30	3 0 (2nd)
1308	10	256/128	9	4 7 (4th)
1311	9	1024/1024	1	Trace (1st)

Dr Goffe the first tissue culture generation in oculated with stool specimens and we chose the first and the last positive stools in all except one case Here the second positive stool culture was sent because the first specimen contained only traces of virus Dr. Goffe titrated the culture fluids which he received and inoculated 0 5 ml into each thalamus of normal cynomolgus monkeys. Usually three monkeys were inoculated with undiluted tissue culture fluid and three also with 1 100 culture fluid Details of all the results will be found in the paper but Table 2 shows the results with three of the sets of excreted viruses and of the vaccine virus hibit some paralytic properties, lesions were present and virus was recovered from the cord The later viruses excreted from all 3 boys had enhanced neurotropic properties, particularly in the case of the strain recovered from 1203 As little as 3 9 logs of virus from this boy paralyzed 2 out of 3 monkeys and produced lesions in all 3 monkeys One of the monkeys receiving the larger dose of this virus had exhibited viremia

Dr Sabin has criticized us for using a tissue culture medium for the primary isolation of virus containing only about 0 1% of bicarbonate—that is Hanks' solution with three times the normal amount of bicarbonate. This might have favored

TABLE 2. RELATIVE NEUROTROPISM OF VACCINE (SABIN) AND EXCRETED TYPE 3 VIRUSES

		Intracerebral Inoculation in Cynomolgus Monkeys			
Source	Days After Feeding	Virus Inoculated TCD_{50} Log_{10}	Paralytic Rate	Lesions	Virus From Cord
Vaccine	—	6 8	0/3	0/3	0/3
		4 8	0/3	0/3	0/3
1205	2	6 8	0/2	0/2	0/2
		4 8	0/3	0/3	0/3
	31	6 9	1/3	3/3	0/3
		4 9	1/3	1/3	1/3
1201	7	7 95	1/3	3/3	1/3
		5 95	2/3*	2/3	2/3
	31	8 15	2/2	2/2	2/2
		6 15	1/3	3/3	2/3
1203	2	7 1	1/3	1/3	1/3
		5 1	0/3	0/3	0/3
	14	5 9	3/3	3/3	3/3†
		3 9	2/3	3/3	2/3

* One monkey died of bacterial meningitis but poliovirus was recovered from cord on 6th day
† Viraemia present in one monkey

First of all, the results with the intracerebrally inoculated monkeys who received vaccine virus were as Dr Sabin has recorded. Neither paralysis nor lesions occurred and virus was not recovered from the cord The viruses excreted on the 2nd day after feeding the boys 1205 and 1203 produced either no effects or minimal effects in the intracerebrally inoculated monkeys That from boy 1201 on the 7th day did however ex- the growth of the particles of virus excreted in the stool which were of a more neurotropic character in view of the effect of an acid pH on the avirulent strain We had however sent to Professor Dick specimens of tissue culture fluids which were re-isolates from the original stool specimens of boy 1203 These kidney cultures were made in Earle's medium which contains more bicarbonate

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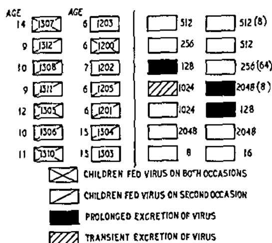


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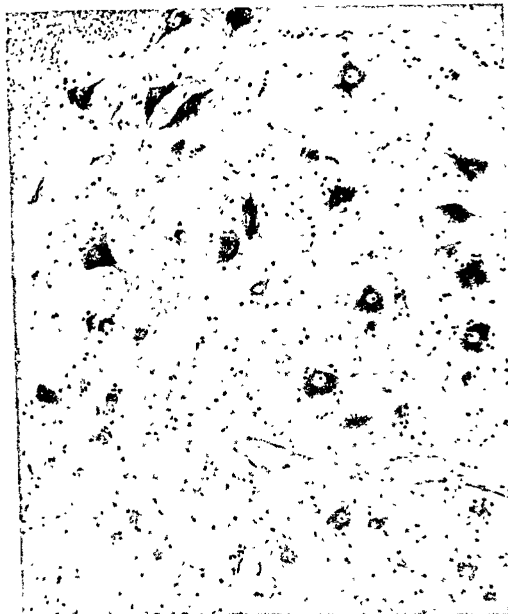


FIG 3 Lumbar cord of monkey killed 28 days after intracerebral inoculation of 6 8 logs TCD_{50} of vaccine virus. Normal.

TABLE 3 COMPARISON OF FIRST AND LAST VIRUS ISOLATIONS FROM CHILD 1203 (DICK)

SOURCE	VIRUS INOCULATED $LOGS_{10}$ TCD_{50}	PARALYTIC RATE	ONSET (DAYS)	LESIONS
2nd day stool	6	0/3	—	—
	3	0/3	—	—
14th day stool	5	2 ? 3/3	17 ? 17 18	2/3
	4	2/3	17 19	3/3
	3	0/2	—	—

Table 3 shows that Professor Dick obtained substantially the same results as Dr Goffe The virus on the 2nd day after vaccine was fed was avirulent at a dose of 6 logs, that on the 14th day caused paralysis after intracerebral inoculation of 4 logs Incidentally, Professor Dick's work was on Rhesus monkeys It would appear that Dr. Sabin's criticism is not the whole answer on the recovery of at least one strain of an excreted virus possessing a fair degree of virulence for monkeys by the intracerebral route

Finally histological examination by Dr Goffe gave results as shown in Figures 3 4, and 5 First the lumbar cord in monkeys receiving vaccine virus (6 8 logs TCD_{50}) shows a normal appearance (Figure 3) Next the lumbar cord of a monkey, paralyzed on the 8th day after intracerebral inoculation with 3 9 logs TCD_{50} from the 14th day stool of boy 1203, shows typical polio lesions (Figure 4) Finally, the lumbar cord of a monkey killed 29 days after intracerebral inoculation of 4 85 logs TCD_{50} from the 14th day stool of case 1202 This shows extensive lesions typical of convalescent poliomyelitis (Figure 5).

Conclusion

What are we to make of the results of this very small trial? In the first place we did not encounter any harmful effects in the boys or their contacts, nor did we find evidence of spread Secondly, we failed to infect as many of the boys as we expected and we obtained antibody responses in only a proportion of those who did become infected Now it is true that we may have used too small a dose of virus, or perhaps the capsules limited the administration of virus or perhaps the previous use of Salk vaccine within two months of virus feeding inhibited a further rise of antibodies.

But our most important finding surely lies in the changed properties of the viruses excreted at the end of the phase of virus excretion compared with that found soon after infection began We have heard a great deal already about the difficulty of comparing vaccine virulence tests conducted by different observers So far there has been little attempt to define an acceptable level of neurotropism of viruses put into vaccines But in the hands of one observer, excreted viruses were certainly very different from vaccine virus and it seems at least as important that this Conference should decide how great a deviation of neurotropism is scientifically and socially acceptable

Until we can be sure about the often-stated lack of parallelism of monkey neurotropism and virulence of polioviruses for man, it seems questionable whether we can accept the existing evidence as an indication of the innocuous character of vaccines such as that tested in this miniature trial



Fig. 5 Lumbar cord of monkey killed 29 days after intracerebral inoculation of 4.85 logs TCD_{50} of virus from feces of case 1202 on 14 days after vaccine. Extensive lesions of convalescent poliomyelitis.

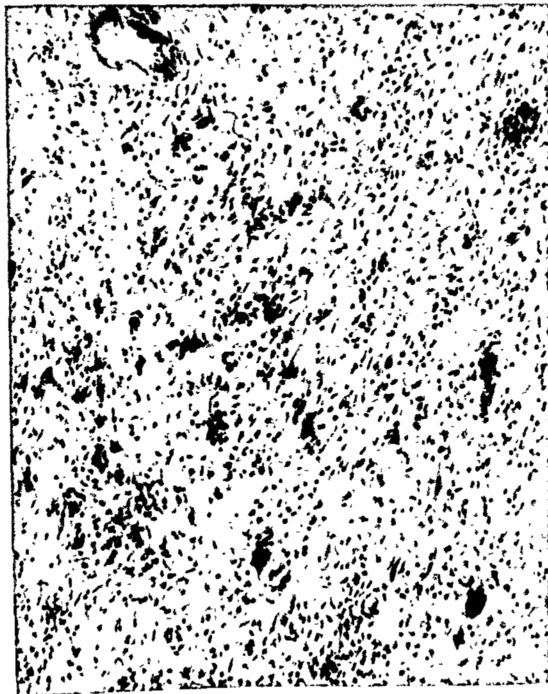


FIG. 4. Lumbar cord of monkey paralyzed 8 days after intracerebral inoculation 3.9 logs TCD_{50} of virus from feces of case 1203 on 11 days. Typical poliomyelitis.

worked, and we realize that we cannot get the answer or the significance of this phenomenon, which we know has been well documented by further studies of this sort in monkeys—that the only significance in this phenomenon we can get is what happens in mass-scale contact infections in the field, and populations with a large number of susceptibles, such as have been described by Professor Smorodintsev, such as will be described by Dr. Voroshilova, Dr Chumakov, and such as I have seen in Czechoslovakia, where 40 to 60 per cent of the children under ten years of age who were contacts, and had no antibody, developed infection by contact without clinical illness

And it is the continued observations of this kind that I think will give us the final answer I frankly do not see that any further studies or any modifications are going to tell us any more about this problem The significance of markers are that they may tell us—I do not know whether they will—that we can differentiate wild strains of certain neurovirulence from others But the study of these markers on the wild strains must be carried out first, in order to have a basis for comparison

DR BODIAN Dr. Sabin has said that all of us are now in agreement that attenuated strains have the potentiality for reversion—at least, that is what I think he said.

He also says that perhaps we ought not pay so much attention to the further laboratory demonstration of this potentiality of reversion, but rather see what happens in the field in large-scale trials

The data which Professor Smorodintsev presented clearly show that reversion, if it occurred had no harmful effects But if we continue with mass trials, without further knowledge, we may introduce this vaccine into a population where perhaps conditions conducive to epidemicity may be prevailing, and we would be testing reversion under somewhat different circumstances from those which perhaps prevailed in Dr Smorodintsev's study

It seems to me that before we propose this sort of thing we should accept a certain degree of responsibility I myself am not clear that we should give up the study of the problem of reversion

There is also a practical aspect to the neurovirulence problem. In testing attenuated strains, before introducing them into individuals as a vaccine, we apply certain tests, the chief test being neurovirulence The WHO recommendation was that intracerebral neurovirulence be a test for the suitability of the strain.

Now, we find that the individuals who have been fed attenuated strains are excreting intracerebrally virulent material and transmitting it. This is going to pose a very serious problem for public health agencies which have the responsibility of licensing live poliovirus vaccine and recommending it for general use, because we are certifying material under standards of neurovirulence which do not apply to the excreted progeny

I think we have to face up to this challenge, since neurovirulence is also a means of discriminating between vaccine strains and the wild strains which may contaminate production lots For example, people who work in poliomyelitis vaccine manufacturing concerns sometimes carry wild strains of poliomyelitis viruses, and these turn up in tissue cultures Absence of intracerebral virulence could be a most convenient limiting characteristic, and since we must hold the line somewhere, I would like to know where we are going to draw it

DR SABIN I think that a certain amount of definition of terms in quantitative thinking is needed in this discussion I think that perhaps if we do that there will be much less disagreement

In the first place, the use of the term reversion, without any sort of quantitative implication, to my mind, does not represent the true state of affairs Because if polioviruses were either virulent or avirulent, meaning that they reverse from avirulent to virulent, then I really think that there would be very great difficulty with the use of a live poliovirus vaccine

But in the case of poliomyelitis—as with every biological property that is tested—the fact is that we are dealing with a spectrum of properties, and that a thing does not revert from white to black, there is a whole spectrum in the range, which has been the basis of the study throughout

When Dr Bodian says now that we agree that there is reversion, I do not see that any of the data presented either by Dr. Stuart-Harris or by

DISCUSSION

CHAIRMAN GEAR The paper presented by Dr Stuart-Harris is now open for discussion

DR DICK I think that Dr Stuart-Harris has put his finger on the crux of the problem

The question I stated quite simply at the opening has been brought up again, and it is this Are vaccine viruses which can spread and have a monkey neurotropism similar to some wild avirulent strains going to prove stable in the field under varying conditions and circumstances?

I appreciate the fact that the answer will come from the field, and if the strains are stable, how easy it will be to prove this when one considers all the coincidences that might arise during large-scale trials? That is the crux of the problem, and I think Dr Sabin will completely agree with me

DR. SABIN: I always like to agree rather than disagree, therefore, let me agree as much as possible with what Dr Stuart-Harris said I have analyzed the data in a previous publication, and I will not repeat them here

Perhaps you will permit me once more to say that what we need to do is to interpret the results of these monkey tests, rather than merely state them

Professor Stuart-Harris made a very important remark. He said that, of course, we use the most sensitive medium for isolating something, but we must also define what the medium is sensitive for, and how we are going to interpret it Let us assume that you have a culture of 100,000 million streptococci that are susceptible to penicillin, and among them you have 100 organisms that are resistant. If you plate them in an ordinary medium you will grow out the mass population which predominates, namely, the streptococci susceptible to penicillin But if you now take a selective medium, a medium that has, let us say, one unit of penicillin in it, it will suppress the others and grow out, if you do not do it on a blood plate, an entire population of resistant organisms to penicillin, at least to one unit

One could interpret that very easily by saying that you have now recovered a population of streptococci that is resistant to one unit of penicillin, when, in effect, it only means that a portion of that population is so resistant

Now, I cannot say that in my own mind I am absolutely certain this is a precise parallel for what occurs in the intestinal tract, but I believe that it is for the following reasons. First, if it were not, then continued propagation in the intestinal tract would not give us the results that we obtained In several instances that I put on record now, and others that I have shown in the tables presented at the first session on the family contact studies of Dr. Gelfand and Dr. Fox, we observe precisely things similar to those Dr Stuart-Harris has reported and also, in that one case which Dr Dick has confirmed, a fact which shows that there is a certain amount of law and order to it

But, on the occasions where we have had an opportunity to study later excretions of the virus, it did not behave this way

Furthermore, I have taken the one most striking instance comparable to the case presented by Stuart-Harris and, to test this hypothesis, I grew the virus in monkey kidney culture, by inoculating it with the 10 per cent stool suspension I also carried out a titration and obtained the population which came out in a terminal dilution which would represent the larger population

Then I tested the two populations, those growing out from putting in the whole 10 per cent suspension of the stool, and that which came out as a terminal dilution.

When I repeated the same test in monkeys, by titration, there was quite a difference in the neurovirulence found with the two specimens There was a reproduction of the results obtained with the 10 per cent stool inoculum, but a very much smaller, a minimal manifestation in a few monkeys that received the culture fluid obtained by the terminal dilution

I do not want to stress it again, because I went into an analysis before that what we are dealing with here is a population of different capacities which can undergo further selection in the culture medium, further selection in the monkey. That is why we have always been concerned with this question during all the years that we have

Then I would like to raise another question about the material which is tested for monkey neurovirulence. If one selects samples at random for testing, one will usually select viruses that represent the majority of the isolates. If they happen to have low neurovirulence like the virus in the vaccine, then one gains the impression that all the isolates are of low neurovirulence. In Dr Smorodintsev's study, for example, in his ten human passages, if he had first screened by *in vitro* characters, and selected for passages only viruses which had shown alterations in their markers, he might have obtained quite different results in his neurovirulence tests than he did by his selection of strains at random.

What we tried to show in our presentation yesterday was that one should screen the isolates first, to see which viruses have changed, and then should concentrate on testing the changed viruses to determine if they are different in neurovirulence from the original vaccine.

Dr Bodian. I am not sure that neurovirulence is the best property to be tested in selecting strains. It is the one that has been used. Most of us who have worked with polio know that there are certain strains with a very high degree of neurovirulence, which are unable to infect chimpanzees or cynomolgus monkeys by feeding so that neurovirulence, in itself, is not a criterion that is absolute in relation to safety. However it happens that many strains that we know to be highly neurovirulent are also highly invasive, such as the attenuated Mahoney strain. What I was trying to bring out is the fact that neurovirulence is the criterion used to select the present candidate strains. If neurovirulence is abandoned we shall have to find another test, if we are going to make any headway in the certification of materials for future lots used in the field.

Dr Sabin referred to the quantitative aspects of reversion. The reason I did not emphasize the quantitative aspects is because I think the significant aspect of reversion is that it occurs at all, and that we cannot prevent it.

Now, if we have the potentiality of change, neurovirulence being only one measure of that change, I say that in the next mass trial in an area where possibly the conditions are ripe for the selection of reverted viral units, we are not able to predict what the outcome will be. This is where our responsibility now lies in interpreting the potentiality for reversion at any quantitive level you please. Since it has been demonstrated that neurovirulence may change with human passage, invasiveness may also be changing, but this property as indicated by viremia, has not been studied adequately.

Chairman Gear. Does Dr. Stuart-Harris have any final comment to make?

Dr Stuart-Harris. I would only comment in relation to Dr Sabin's mention of the susceptibility of the human in regard to this question of virulence. We have focussed our attention so rigidly on the parasite in this connection, that we are very apt to forget that virulence is merely an attribute of a parasite and it is the attribute which you measure by application to the host. I do not know what the host susceptibility to the various types of polioviruses which we have heard about here is, and I am quite sure that susceptibility is not the same in one part of the world as in another.

others who have reported previously, and carried out work on much larger material, show that it is a consistent or progressive pattern

Therefore, merely to use the word reversion without giving some indication of the quantitative aspects as to what it reverts, or what it means, is not a correct expression of the situation

Now, this thing, as everyone agreed, was a problem that had to be studied, and was studied from the very beginning It is not a thing that we suddenly now agree on, and now discover This is a problem that has been studied in a quantitative manner to find out the degree of the change in the spectrum.

The assumption has arisen from the data that have been collected that the more active a material is to begin with in large numbers of cases, not in any one individual case, the greater the frequency of greater activity farther along on the spectrum, the less the activity, the less the frequency

In any one case you cannot prove it; and that is the basis for the continued work over a period of years to obtain the material with the least amount of residual neurotropisms

As far as I can see, if we keep this quantitative aspect in our mind and we do not say that the viruses revert to virulence, I believe we will be thinking about it much more clearly because there is not a single instance in which reversion to full neurovirulence has occurred

And, furthermore, the evidence is that what we are looking for, what we are checking, what we are finding, is what happens in the monkey, and does not represent the major population in the human alimentary tract which is passed directly from one human alimentary tract to another human alimentary tract, without preliminary passage and selection in the monkey nervous system

DR. ARMSTRONG. I would like to call attention to a virus that I discovered some twenty years ago, sent to me from Lansing Michigan The virus was infectious to mice, cotton rats, pack rats, and hamsters Now, I might have felt at that time that I had the greatest killer of all times, because I had a virus which attacked all these different species. This virus is now known as Type 2 poliovirus, the least virulent of the three types; yet it is causing immunity in a large percentage of the population.

I feel that by placing too much stress on these delicate tests—putting the material artificially and directly into the lumbar cord of monkeys—is just making trouble for ourselves We must, I think, pay some attention to a certain amount of intracerebral susceptibility, i e, adaptability of the viruses to the species, but that does not mean, necessarily, that we must go on to ever more delicate tests

I think, also, that in the first feeding with live virus vaccines we need have very little concern with reversion, but after secondary natural transfers there may be great danger of confusion with naturally occurring wild strains. The only method of minimizing this confusion is to vaccinate in nonepidemic times—February and March—and not in the face of epidemics or in the summer.

DR MELNICK: Dr Sabin has indicated that one should consider aspects of reversion, and I certainly agree with him There are ways of measuring this, as indicated in our presentation yesterday For example, we started with his Type 3 vaccine strain, which contained 100 monkey-paralyzing doses per ml by the intraspinal route, and after passage in man we recovered virus which contained 1,000,000 monkey paralyzing doses—or a thousandfold increase in monkey neurovirulence This increase was paralleled by a change from negative to positive after intracerebral inoculation.

Dr Sabin has also raised the question of selection of particles by the spinal cord of the monkey test, namely, that only a very small proportion of these particles may multiply and cause paralysis However, in some of our recent studies with the specimens that were collected from the vaccinated children in Mexico City, it was found that as few as ten particles present in the original rectal swabs were capable of producing paralysis in a monkey when inoculated intraspinally To us, this indicates that the majority of the particles are neurotropic If we are going to talk about a monkey selecting from these ten particles, then I think selection has little meaning If neurotropic mutants arise in the course of testing virus isolated from vaccinated children, then one would expect them to arise with the same frequency when the vaccine virus is inoculated into monkeys, which was not the case.

seemed to have been exposed to Type 2. Apparently, hardly any strains of poliovirus have been circulating in Sweden after 1953, and as a consequence we have built up a population of highly susceptible children.

One can imagine what might happen, if a virulent, epidemic Type 1 strain were introduced in such a population. Would, in that event, immunization with inactivated vaccine provide a satisfactory protection? We could not feel confident that this would be the case, and what has later been reported, particularly from Israel, has only confirmed our suspicions. The Israeli experience in the epidemic year 1958 indicates that immunization with inactivated vaccine does not give adequate protection against the massive exposure in the course of a severe epidemic.

In conclusion, we deemed it highly desirable to reinforce the immunity obtainable with inactivated virus, particularly to Type 1. At the same time the exceptionally low level of natural immunity in the population called for great caution in the use of live virus. We had to accept as acknowledged facts that all attenuated strains at present available have some residual neurovirulence, that they tend to change some of their properties when multiplying in the human intestinal tract, and that they spread from vaccinees to contacts.

In order to minimize the risk of untoward reactions we decided to require that all persons who might be exposed, vaccinees and contacts alike, should have been successfully immunized with inactivated vaccine, before live virus was fed. In order to keep spread of infection under best possible control we decided to accept as volunteers only such families in which no children attended schools, nursery schools, or day nurseries, and to confine the tests to winter and early spring when conditions for a wider dissemination of virus are poor.

We finally selected a group of 20 families among the clientele of one Children's Health Center in Stockholm, each family with at least two children in the pre-school age. We started by taking blood samples from all members of the household, everybody was given two shots of inactivated vaccine and the result was checked serologically. Persons who had not reacted to Type 1 were revaccinated. When all household members had Type 1 antibodies, virus was fed to the youngest child in the family, the index child. Virus excretion in the stools was studied in all members of the household, samples being collected twice weekly, later weekly, as long as any one member excreted. Bleedings were taken about 2 months after the feeding of the index child, and again 8 to 10 months later. Following this, every household member was challenged with live virus, excretion again studied and bleedings taken about two months later.

From the serologic tests and the reactions to administration of live virus we concluded that all of the 44 children in this group had had no previous experience of live Type 1 virus, as was apparently true also of 10 of the 44 adults. Thus, our group consisted of 31 adults with preexisting natural immunity and 54 persons without such immunity to Type 1. At the time of the second feeding two newborn babies had increased the latter figure to 56.

The first 5 index children were fed 10^6 ID_{50} of virus and became promptly infected. The next 4 were given $10^{4.5}$ ID_{50} with one failure. This child was immediately refed 10^6 ID_{50} and then became infected. After this experience we used consistently a dose of 10^6 ID_{50}, also in the chal-

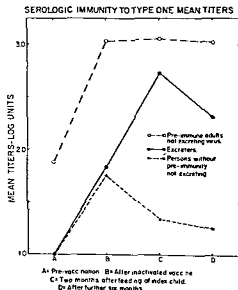


Fig. 2. Serologic immunity to Type 1. Geometric mean titers.

2. VACCINATION WITH ATTENUATED POLIOVIRUS TYPE 1, THE CHAT STRAIN

S. GARD, MARGARETA BÖTTIGER, AND R. LAGERCRANTZ

Department of Virus Research, Karolinska Institutet; the State Bacteriological Laboratory; and Department of Pediatrics, Karolinska Hospital, Stockholm, Sweden

DR GARD *(presenting the paper)* Vaccination with attenuated Type 1 poliovirus is currently being tested in Sweden in controlled, small-scale trials. Together with Dr. Bottiger and Lagercrantz, I started the first field trial with Koprowski's CHAT strain in the fall of 1957 Soon after that Wesslén embarked upon an experiment with Sabin's LSc 2ab strain in an institution for mentally retarded children Finally last winter a larger trial along the same lines as our first test was initiated The aim of this report is to describe our first trial and summarize the experiences gained

As our approach to the live virus vaccination problem seems to be slightly different from what has so far been described at this meeting, I would like in a few words to present the background against which our attempts have to be viewed

Figure 1 shows the occurrence of paralytic poliomyelitis in Sweden during the last five years In 1953 we had a great epidemic of some 3,400 cases; following which a progressive, rapid reduction in annual rates has set in In 1958 the number of cases was only 3 per cent of the 1953 total

Unfortunately we cannot ascribe this development to the effect of vaccination, as our vaccination program was initiated first in the spring of 1957 As a matter of fact Sweden might well serve as a negative control in attempts at evaluation of the American experiences of the vaccine

The same phenomenon, virtual disappearance of poliomyelitis in the 5-year period following a nationwide epidemic has been observed before in Sweden It happened after our first big outbreak in 1905 and again after the very big epidemic in 1911-1912.

Looking for an explanation we have surveyed the under 5-year age groups for neutralizing antibodies In the spring of 1958 two groups of each

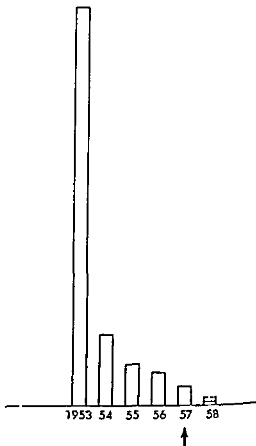


FIG 1 Number of cases of paralytic poliomyelitis in Sweden 1953-1958 Arrow indicates initiation of immunization with inactivated virus vaccine

about 100 children, one from the capital Stockholm and one from a small provincial town in Central Sweden, were studied Among those 200 children none showed evidence of previous experience of Types 1 and 3, and only one child

tralization titers The figures recorded represent cumulative percentages of individuals excreting virus for less than 1, 2, 4, 8, or 16 weeks

Starting with category III, all became infected If you compare the serum titer classes, you will find an obvious trend, reflected also in the average duration of excretion in the last column of the table There seems to be a significant inverse

TABLE 1 BREAKDOWN OF RELATIONSHIP BETWEEN DURATION OF EXCRETION AND ANTIBODY TITER
I· ADULTS WITH PRE-EXISTING NATURAL IMMUNITY, II· CHILDREN WITH A SINGLE PREVIOUS EXPOSURE TO LIVE TYPE 1 VIRUS III. PERSONS WITHOUT PREVIOUS EXPOSURE TO LIVE VIRUS

	Titer	No. of persons	% excreting virus for less than					Mean excretion period
			1	2	4	8	16 weeks	
I	<10	0						
	10-50	4	75	100				0.25
	250-1250	15	93	93	100			0.15
	6250-31250	11	91	100				0.09
II	<10	0						
	10-50	8	13	88	100			1.13
	250-1250	19	21	95	100			0.84
	6250-31250	0						
III	<10	7	0	0	29	43	100	7.43
	10-50	34	0	3	29	76	100	5.35
	250-1250	15	0	13	53	73	100	4.53
	6250-31250	0						

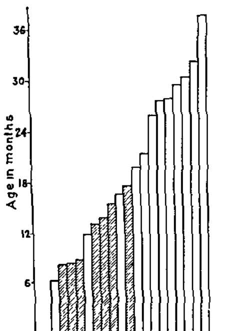


FIG 5 Index children age at time of feeding Hatching indicates spreaders

correlation between titer at the time of feeding and duration of excretion A similar trend may be traced in groups I and II, although much larger numbers have to be studied, before any conclusions may be drawn

An important fact is the clearcut differences between the three categories, and particularly interesting that between the naturally immunes with presumably several exposures to the virus and those with one single previous exposure In the former group only 3 out of 30 became infected and short time excreters, whereas in group II no less than 22 out of 27 excreted virus Antibody titers alone can apparently not explain this difference

Spread of infection As already mentioned 8 out of 35 presumably susceptible contacts became infected in the first feeding experiment, all of them children Fig 5 illustrates one of the conditions that seems to favor spread of virus It shows the 19 index children in order of age at the time of feeding, from 6 months to about 3 years Hatched bars represent spreaders It is a conspicuous fact that all of the 7 spreaders are found among the 10 children less than 18 months, none among the 9 children above this age

lenge test The index children excreted virus for from one to twelve weeks, average 5 6 weeks Of altogether 35 presumably susceptible contacts 8 became infected and excreted virus for comparable periods of time

Serologic tests (Fig 2). The first plots (A) represent the pre-immunization status The pre-immune adults had thus a geometric mean titer of close to 1 100, while no antibodies were detected in presumably susceptible individuals (less than 1 10)

Upon administration of inactivated vaccine the adults responded fairly well (B), and the titers remained on the same high level throughout the observation period, which now is about 14 months Also the non-immunes responded well with mean titers not far from the pre-immunization level of the first group.

However, in the non-immunes who did not excrete virus in the first phase of the experiment a considerable drop in titer was observed 2 months later (C) and a further slight decline after 8 months (D) This resembles the type of curve you find in experimental immunization of animals a fairly rapid initial drop, later on the curve levels off.

In children excreting virus we found a considerable rise in titer after 2 months, the average level approaching that of pre immunes boostered with inactivated vaccine However, a significant drop in titers was observed 8 months after feeding, although there was still a difference of about one log from the non-excreters

Not shown in the diagram are the responses after the challenge feeding The adults with natural immunity showed no response, if anything there was a slight decrease in titers Children with one previous experience of live virus reacted with considerable rises, the mean titer of the group reaching 3 3 log, clearly above the level of the naturally immunes Those without previous experience, finally, responded exactly as did the previous group on first feeding

Resistance to oral infection Fig. 3 shows degree of resistance in relation to antibody titer at the time of feeding. Cross-hatching represents those in which no excretion was demonstrable (= resistance); single hatching represents short-time excreters, less than two weeks (= partial resistance); white areas represent those who excreted for two weeks or more (= *susceptibility*) Results are plotted against serological titer at the time of feeding, 0 = less than 1 10 the highest concentration tested, 1 = 1.10, 2 to 6 represent 5-fold steps up to 31,250

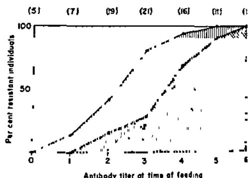


Fig 3 Resistance to oral infection in relation to antibody titer at time of feeding Cross hatching = no excretion Single hatching = short time excretion Numbers of individuals in brackets on top of the diagram

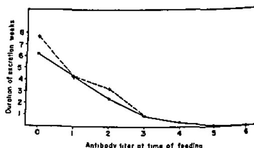


Fig 4 Average duration of excretion in *relation to antibody titer at time of feeding*

Fig 4 shows average excretion periods plotted against titers Both these diagrams show a close correlation between serological titer and degree of resistance, expressed in terms of *duration of* excretion

In Fig 2, however, it was shown that the level of serologic immunity was distinctly correlated to the previous infection history, and for that reason a further break-down of the results is necessary This is done in Table 1. Three categories are recorded separately: I = adults with pre-existing natural immunity; II = children with one single experience of live virus 8 to 10 months previously; and III = persons without previous experience of live virus They are *further classified in 4 groups according to neu-*

tralization titers. The figures recorded represent cumulative percentages of individuals excreting virus for less than 1, 2, 4, 8, or 16 weeks

Starting with category III, all became infected. If you compare the serum titer classes, you will find an obvious trend, reflected also in the average duration of excretion in the last column of the table. There seems to be a significant inverse

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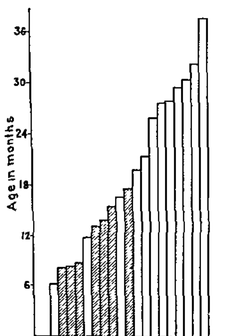


FIG 5 Index children age at time of feeding Hatching indicates spreaders

correlation between titer at the time of feeding and duration of excretion A similar trend may be traced in groups I and II, although much larger numbers have to be studied, before any conclusions may be drawn

An important fact is the clearcut differences between the three categories, and particularly interesting that between the naturally immunes with presumably several exposures to the virus and those with one single previous exposure In the former group only 3 out of 30 became infected and short time excreters, whereas in group II no less than 22 out of 27 excreted virus Antibody titers alone can apparently not explain this difference

Spread of infection As already mentioned *8 out of 35 presumably susceptible contacts became infected* in the first feeding experiment, all of them children Fig 5 illustrates one of the conditions that seems to favor spread of virus It shows the 19 index children in order of age at the time of feeding from 6 months to about 3 years Hatched bars represent spreaders It is a conspicuous fact that all of the 7 spreaders are found among the 10 children less than 18 months, none among the 9 children above this age

The significance of this observation has been fortified in our current trial on 95 families All index children in this experiment were born in 1954 (4 years +) Of the 95 index children, 32 belong to one-child families, while 63 had a *total of 73 presumably susceptible siblings Of* these 63 children only 3 spread virus to contacts

Stability of the virus Finally we are studying the question of "genetic" changes in the virus propagating in the intestinal tract We started with the *d* marker So far results are available from 14 of 19 index children and all 8 contact infections from the first feedings. Of 14 index children 3 excreted virus of clearcut d^{+} character and one a virus retaining the *d* marker but giving large plaques instead of the typically small plaques produced by the original vaccine virus Of the 8 contacts 4 excreted d^{+} virus In all except one case the first isolate had retained its *d* marker, d^{+} variants appearing later In one case, systematically studied, the d^{+} variant appeared in the second week and remained for the duration of the excretion period

Only a very limited number of neurovirulence tests have been carried out so far The original virus, inoculated intracerebrally in a dose of 10^{6} ID_{50} produced no paralysis in four monkeys but typical histopathological lesions in one Of excreted strains we have first chosen those showing the most pronounced d^{+} characters. Tests are now completed for two strains only Neither strain produced paralysis but in one monkey slight lesions of a possibly specific nature were observed at the site of inoculation. These tests are being continued

Conclusions. We consider the results of the first trial promising. Our material is, of course far too small for an evaluation of the safety of the procedure. I am almost embarrassed to *have to confess that we have not observed any* untoward reactions whatever It has pleased us particularly that the spread of infection remained limited and that we found indications how further to minimize and control it

Our plans for the future do not entail either inactivated or live virus but a combination of both Thus, an early immunization with inactivated virus preferably incorporated in the triple *vaccine (TPD) that should be given anyway* In order to minimize the risk of spread of infection the follow-up with live virus should be delayed till after the second year of life It is to be hoped that the inactivated vaccine will provide sufficient protection for the intervening period.

We feel that for the time being we don't have to worry much about any other type than Type 1

3. A SMALL-SCALE TRIAL ON VACCINATION AND REVACCINATION WITH LIVE ATTENUATED POLIOVIRUSES IN THE NETHERLANDS

PROFESSOR J. D. VERLINDE AND DR. J. B. WILTERDINK

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PROF. VERLINDE *(presenting the paper)*: Since the immediate results concerning alimentary infection, development of antibody and neuropathogenicity of viruses excreted by individuals vaccinated with attenuated poliovirus vaccine in the Netherlands have been published, I shall only briefly refer to this subject with reconsideration of the results obtained.

Problems of immunity to reinfection, however, will be considered more in detail

Epidemiological Introduction on Cyclic Prevalence of the Three Types of Poliovirus in the Netherlands in Relation to Active Immunization

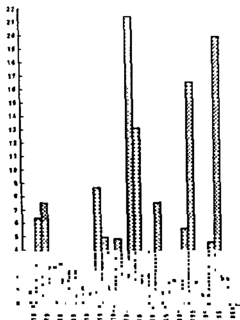


FIG 1 Notified cases of poliomyelitis in the Netherlands, morbidity per 100,000

Major outbreaks of poliomyelitis in the Netherlands tend to occur every four to five years (Fig 1). The epidemic years are characterized by a predominance of Type 1, as shown in Fig. 2 (approximately 90 per cent of the strains isolated). Type 2 tends to an approximately equal distribution during all years, whereas Type 3 shows a tendency of fluctuation almost similar to that of Type 1.

Most of the epidemic years are preceded by a year with relatively increased incidence A pre-epidemic year has most likely to be regarded as a precursor of an epidemic The relative incidence of Type 1, which was responsible for the 1956 outbreak, was already increasing during the last quarter of 1955, reached its peak in the summer of 1956, and then declined rapidly during 1957 (Fig 3)

The age distribution of antibody shows that immunologic maturity in the Netherlands has been reached at the age of approximately 15 years for all three types (Fig 4) This means, with regard to a major spread of Type 1 virus with four year intervals, that every four years 20 per cent of the homotypic non-immune individuals are experiencing a Type 1 infection, whereas the chance of natural exposure during inter-epidemic years may be relatively low. As a matter of fact, Type 1 virus was shown to be widely spread among the population in the epidemic year 1956[1] whereas it was found to be less widespread in 1957 and considerably less in 1958[2]

Continuous circulation of poliovirus among the entire population would have the advantage of continued natural exposure and, consequently, of converting sero-immunity of limited duration as produced by killed virus vaccine into immunity of prolonged duration If continued re-

FIG 2 Fluctuation in the prevalence of types of poliomyelitis virus virologically positive patients.

FIG. 3 Fluctuation in the prevalence of types of poliomyelitis virus virologically positive patients.

search should confirm the observation of cyclic increase and decline of the circulation of polioviruses, particularly that of Type 1, the chance of converting acute immunity into chronic immunity (Paul, 1956)[8] by natural exposure of vaccinated individuals will be low during inter-epidemic periods It then remains to be seen whether the application of killed virus vaccine at the beginning of an inter-epidemic period will induce a sufficient level of immunity for covering this period up to the next period of increased circulation of Type 1 virus

Alimentary Infection and Development of Antibody after Feeding Live Virus Vaccine

Approximately 200 children and adults have been fed 100,000 to 1 million tissue culture infection doses of all three types of poliovirus, using the strains LSc 2ab (Type 1), P 712 CH 2 ab (Type 2) and Leon 12 ab (Type 3) selected and supplied by Dr A. B. Sabin. The strains were fed separately in the sequence Type 1, Type 3 Type 2, with intervals of three weeks, and in a teaspoonful of cream milk. At least 10 children were triple negative, at least 80 individuals had no pre-existing antibody to one or two types The first trial started in May 1957, shortly before the polio season, the second trial started in December 1957, after the polio season.

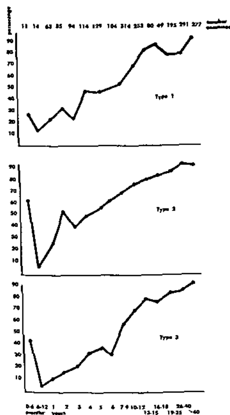


Fig 4 Age distribution of poliomyelitis virus neutralizing antibodies in 2,073 individuals from the general population of the Netherlands (1957)

Stool specimens were collected once a week, the first one immediately prior to feeding, in order to be sure that no interfering virus was present

Among the individuals without pre-existing antibody, fecal excretion of virus was demonstrable in children at a percentage roughly twice as high as in adults (Table 1) The percentage of individuals with demonstrable fecal excretion was considerably lower in those with pre-existing antibody In this group also, the incidence of demonstrable fecal excretion was roughly twice as high in children as in adults. In those who may have excreted the virus for short periods we may have missed the virus.

Two of the children with pre-existing antibody who excreted virus, had received two injections of Salk vaccine, the last injection having been given four and six weeks prior to the ingestion of the live virus vaccine In all other individuals, pre-existing antibody was a result of previous natural exposure

In the majority of the individuals without pre-existing homotypic antibody, fecal excretion of virus was demonstrable for an average period of two to six weeks, whereas this period was one to two weeks on the average in individuals with pre-existing homotypic antibody (Table 2) The duration of the alimentary infection tended to be longer in children than in adults. Figure 5 shows more clearly the duration of alimentary infection. We can see here that in the majority the individuals without pre-existing homotypic antibody fecal excretion averaged two to six weeks, whereas this period was only one to two weeks on the average in individuals with pre-existing antibodies

The difference between children and adults as to the percentage of demonstrable fecal excretion

TABLE 1. VIRUS EXCRETION IN VACCINATED INDIVIDUALS WITHOUT AND WITH PRE-EXISTING ANTIBODY

Pre-existing Homotypic Antibody (pH Test)	Number and Percentage of Individuals With Demonstrable Excretion of Virus							
	0-14 Years of Age				14 Years of Age and Older			
	Type 1	Type 2	Type 3	All Types	Type 1	Type 2	Type 3	All Types
negative	29/38 77%	28/36 78%	43/45 96%	100/119 84%	2/4 50%	1/4 25%	1/1 100%	4/9 44%
positive	8/26 31%	9/24 37%	8/15 53%	25/65 38%	2/10 20%	1/10 10%	3/13 23%	6/33 18%

TABLE 2 DURATION OF FECAL EXCRETION OF VIRUS

Pre-existing Homotypic Antibody (pH Test)	Number of Days and Average Duration of Demonstrable Alimentary Infection					
	0-14 Years of Age			14 Years of Age and Older		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
negative	5-33 19	5-48 19	7-44 26	10-11 11	8 8	11 11
positive	5-32 12	4-34 16	5-21 9	8-11 10	10 10	8-21 15

of virus and the duration of alimentary infection indicates that the attenuated viruses multiply more readily in the alimentary tract of children than of adults, and, as could be expected, more readily in homotypic non immune than in homotypic immune individuals.

The amount of virus excreted per gram of stool was highest during the first or second week after ingestion of the virus, and it tended to decline gradually (Fig. 6). The line which is inserted here means the median character during the successive weeks of excretion of virus, each dot represents one fecal specimen from which is given the amount of virus per gram of feces

Neutralizing antibody to all three types developed in all the children and adults without pre-existing homotypic antibody and in whom alimentary infection has been demonstrated by fecal excretion of virus Moreover, all the children and all but one of the adults without pre-existing homotypic antibody and without demonstrable alimentary infection have also developed antibody. Finally, the vast majority of those who have not been examined for fecal excretion of virus has shown serologic evidence of infection (Table 3).

The overall immunizing effect in children without pre-existing antibody was 100 per cent for Types 1 and 3 and 90 per cent for Type 2. The lower antibody response percentage for Type 2 might be due to the fact that Type 3 frequently produced a *long-lasting alimentary infection*, which may have interfered with the subsequent Type 2 infection in some individuals. The inter-

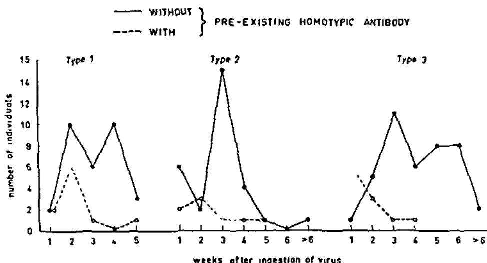


FIG 5 Duration of fecal excretion of virus in individuals

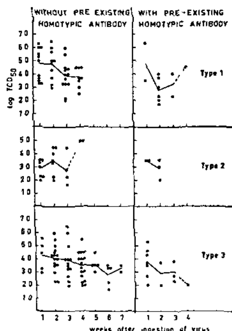


FIG 6 Amount of virus excreted per gram of stool (expressed in log 10 TCD_{50}) by individuals vaccinated with live attenuated poliomyelitis virus vaccine

val between the different feedings might, therefore, perhaps better be extended to at least four weeks

The percentage of individuals responding with a *four-fold or greater rise in antibody titer in* those with pre-existing homotypic antibody was unexpectedly high, viz 67 to 81 per cent in children and 48 to 57 per cent in adults (Table 3)

Persistence of Sero immunity and Resistance of the Alimentary Tract after Natural Exposure and after Ingestion of Live Virus Vaccine

There are marked differences between children and adults as to the development of both alimentary infection and antibody

1 Demonstrable fecal excretion of virus occurred in children without, as well as in those with, pre-existing homotypic antibody in percentages approximately twice as high as in the corresponding groups of adults

2 The duration of the alimentary infection was, at least as far as individuals without pre existing homotypic antibody are concerned, approximately twice as long in children as in adults

3 Homotypic antibody developed in children without, as well as in those with, pre-existing antibody in percentages approximately 1 5 times as high as in the corresponding groups of adults

These observations indicate that sero immunity

TABLE 3 DEVELOPMENT OF NEUTRALIZING ANTIBODY IN INDIVIDUALS WITHOUT PRE-EXISTING ANTIBODY AND FOURFOLD OR GREATER RISE IN TITER IN THOSE WITH PRE-EXISTING ANTIBODY

Age Group	Pre-existing Homotypic Antibody (pH Test)	Type	Number of Individuals in Whom Antibody Has Been Produced: Alimentary Infection Demonstrated	Alimentary Infection Not Demonstrated	Alimentary Infection Not Examined	Total	Immunizing Effect
0–14	negative	1	14/14	3/3	3/3	20/20	100%
	negative	2	11/11	5/5	2/4	18/20	90%
	negative	3	24/24	1/1	3/3	28/28	100%
>14	negative	1	3/3	1/2	16/23	20/28	71%
	negative	2	1/1	3/3	27/29	31/33	94%
	negative	3	1/1		17/25	18/26	69%
0–14	positive	1	7/8	10/14	3/4	20/26	77%
	positive	2	7/7	12/17	3/3	22/27	81%
	positive	3	5/9	7/8	2/4	14/21	67%
>14	positive	1	2/2	3/8	40/69	45/79	57%
	positive	2	1/1	5/9	29/63	35/73	48%
	positive	3	1/3	4/9	38/65	43/77	56%

as well as resistance of the alimentary tract, both as a result of natural exposure are declining, that re-infection of the alimentary tract, although *usually of limited character, may occur, and that* such a reinfection may have a booster effect on the antibody level

When examining the rise in antibody titer in *vaccinated individuals with pre existing* homo-

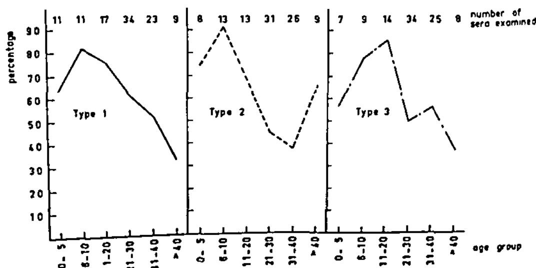


FIG. 7. Rise in antibody titer (pH test) after administration of live virus vaccine in individuals with pre-existing homotypic antibody in relation to age.

typic antibody in relation to age, it is striking that the highest percentages of individuals responding with a rise in antibody titer occur in the age-groups of six to 10 for Types 1 and 2 and in the age-group round 10 for Type 3 (Fig 7) This might indicate that individuals of these age-groups possess the highest relative susceptibility to immunity-reinforcing reinfections, in other words, that immunity of the alimentary tract as a result of natural exposure, which in the Netherlands predominantly occurs in the 0 to 5 year age-group, has declined within approximately five years so far that a limited alimentary reinfection is possible.

The higher resistance of the alimentary tract of adults than of children does not seem to be due to acquired specific immunity only The relatively low incidence of demonstrable fecal excretion of virus in adults without pre-existing homotypic antibody probably indicates that their alimentary tract is less susceptible to poliovirus infection than that of children This assumption is supported by an analysis of the development of antibody in individuals without pre existing homotypic antibody after ingestion of live virus vaccine in relation to age The highest percentages of individuals responding to administration of live virus vaccine with the development of antibody occur in the younger age-groups, which is most striking for Types 1 and 3 (Fig 8). We have observed that adults without pre-existing antibody may fail to develop demonstrable alimentary infection and antibody after repeated ingestion of live virus vaccine, even in amounts of 10 million tissue culture infective doses

It might be provisionally concluded that a long term immunity against poliomyelitis is primarily based on repeated natural exposure and secondarily on a non-specific resistance of the alimentary tract, which tends to increase with age

Resistance of the alimentary tract does not seem to be entirely dependent on the level of circulating antibody, since demonstrable fecal excretion of virus has been found to occur in individuals with both relatively low and relatively high homotypic antibody levels (Table 4) I must add, however, that most of the individuals with a high level of circulating antibody failed to excrete virus

There does not seem to be any reason for assuming that immunity produced by the ingestion of live virus vaccine would be highly dissimilar from that produced by natural exposure This may be evident from the results in 20 children, who have been re-fed the same dose of live Type 1 virus vaccine either 15 or 22 months after primary feeding Stool specimens were collected

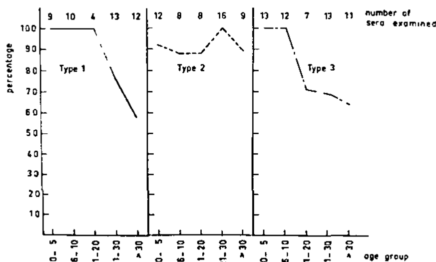


Fig 8 Development of antibody (pH test) after administration of live virus vaccine in individuals without pre existing homotypic antibody in relation to age

TABLE 4. ALIMENTARY INFECTION AND TITER OF PRE-EXISTING HOMOTYPIC ANTIBODY

Type	Titer of Pre-existing Antibody (pH Test) Original Dilution of Serum)	Demonstrable Excretion of Virus			No Demonstrable Excretion of Virus
		Number of Individuals	Duration (Days)	Average Amount of Virus Per Gramme of Stools (TCD_{50} Log_{10})	Number of Individuals
1	1/4	3	8–8–32	5.0–1.7–3.7	5
	1/8	1	8	3.5	2
	1/16	0			4
	1/32	2	8–11	2.3	3
	1/64	3	5–7–11	3.0	1
	1/128	1			4
	1/256	0			1
	1/1024	1	16	2.3	2
2	1/4	1	14	3.5	3
	1/8	2	7–34	3.5	5
	1/16	3	8–10–21	2.0–3.0	0
	1/32	1	4		3
	1/64	1	27	3.5	5
	1/128	0			5
	1/256	0			2
	1/512	0			1
	1/1024	0			3
3	1/4	1	12	3.5	2
	1/8	1	5		1
	1/16	3	5–7–8	2.0	7
	1/32	1	5	4.6	2
	1/64	1	24	2.3	4
	1/128	0			1
	1/256	2	6–14	3.7	0
	1/1024	2	5–21	3.7	0

from them twice a week and blood samples were collected immediately before revaccination and three weeks later. These two serum samples were stored at –20°C and examined for antibody in the same pH test together with two serum samples collected from the same children immediately prior to the first feeding and three weeks later. Hence, four serum samples of each child were examined simultaneously: (1) pre-vaccination serum, (2) post-vaccination serum; (3) pre-revaccination serum; (4) post-revaccination serum.

Unfortunately, only three originally triple negative children were in the group. Although a considerable fall in antibody level had occurred during the period between the first and the second feeding, fecal excretion of virus could either not be demonstrated or lasted for a few days only, whereas the amount of virus excreted per gram of stool was almost negligible (Fig. 9).

Children who originally had no Type 1 antibody, but who possessed Type 2 and or Type 3 antibody behaved almost similarly after re-ingestion of live Type 1 virus vaccine (*Fig. 10*). Finally, five out of 14 children who originally had Type 1 antibody and antibody to one or both of the other types, were found to excrete small amounts of virus for a short period.

Revaccination usually had a booster effect on the level of homotypic (Type 1) antibody, and

in several children also on the level of heterotypic antibody.

Neurovirulence of Polioviruses Excreted by Vaccinated Individuals

Dr Sabin [*] has pointed to the importance of the method of intraspinal inoculation; isolates that fail to exhibit spinal activity following inoculation through the third intervertebral space above the level of the iliac crests showed a definite spinal activity when inoculated through the fourth space (Table 5)

None of the 20 monkeys inoculated through the third space showed any spinal activity as measured by clinical signs of illness

When the same strains had been inoculated into the fourth space above the iliac crests, a considerable number of monkeys have indeed shown paralysis

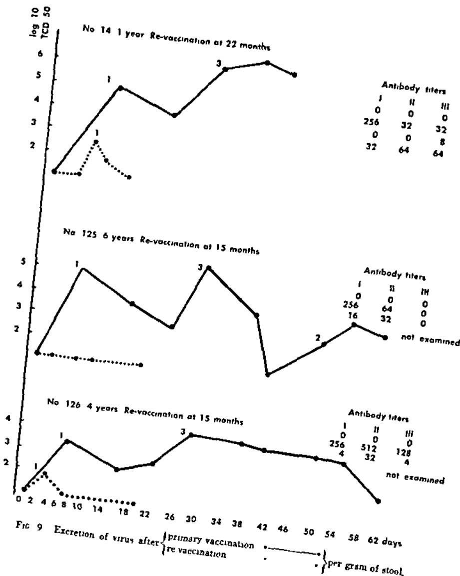


Fig 9 Excretion of virus after {primary vaccination, re vaccination} per gram of stool

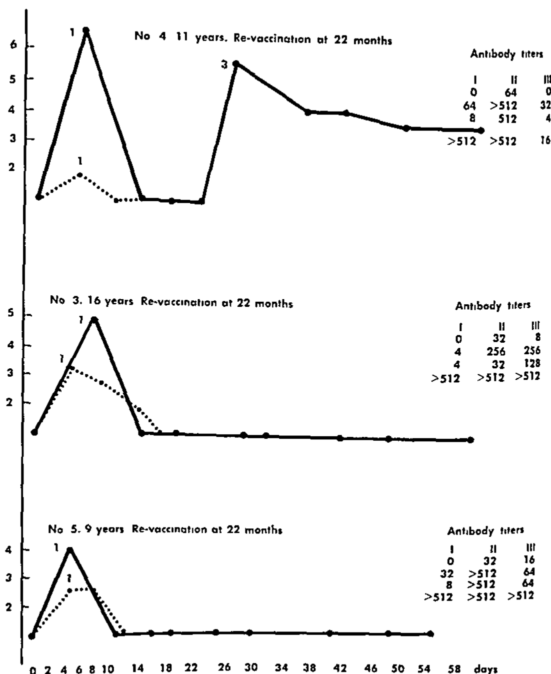


Fig 10 Excretion of virus after {primary vaccination —— / re vaccination ····} per gram of stool

Table 6 Neuropathogenicity of Polioviruses Excreted by Vaccinated Individuals

Type	Virus Isolated After Ingestion (Week)	Intraspinal Inoculation: Number of Strains	Intraspinal Inoculation: Dose (TCD_{50} Log_{10})	Intraspinal Inoculation: Paralytic Incidence	Intraspinal Inoculation: Dose	Intraspinal Inoculation: Paralytic Incidence	Intracerebral Inoculation: Number of Strains	Intracerebral Inoculation: Paralytic Incidence: Dose 7 9—8 3	Intracerebral Inoculation: Paralytic Incidence: Dose 6 9—7 3	Intracerebral Inoculation: Paralytic Incidence: Dose 5 9—6 3
1	1st	8	5 5—8 5	1/16	4 5—7 5	0/16				
	2nd	6	6 2—7 5	3/12	5 2—6 5	0/12				
	3rd	4	6 4—7 0	3/8	5 4—6 0	0/8	2	0/4		
	4th	1	6 1	0/2	5 1	0/2				
2	1st	4	5 3—7 1	0/8	4 3—6 1	0/8				
	2nd	4	5 4—7 7	1/8	4 4—6 7	0/8				
	3rd	2	5 3—7 4	1/4	4 3—6 4	0/4				
	4th	1	7 4	1/2	6 4	0/2				
3	1st	7	6 6—8 0	1/14	5 6—7 0	0/14				
	2nd	3	6 6—7 4	1/6	5 6—6 4	0/6	1	0/2		
	3rd	9	6 0—7 5	8/18	5 0—6 5	5/18	3	7/8	4/6	0/6
	4th	0								
	5th	3	6 9—7 5	1/6	5 9 6 5	0/6				
	6th	1	6 8	0/2	5 8	0/2				
	7th	1	6 2	0/2	5 2	0/2				

Table 5. Paralytic Incidence in Cynomolgus Monkeys Following Intraspinal Inoculation of Attenuated Polioviruses Excreted by Vaccinated Individuals Through the Third and the Fourth Intervertebral Space Above the Iliac Crests

Type	Dose (TCD_{50} Log_{10})	3rd Space Paralysis	4th Space Paralysis
1	5 6—6 5	0/8	3/8
2	6 4—6 7	0/6	3/6
3	6 1—6 5	0/6	2/6

Non-progressive paralysis following intraspinal inoculation of cynomolgus monkeys with high doses of first passage monkey kidney cell culture isolates from stool samples of vaccinated individuals occurred in 18 per cent of the monkeys inoculated with Type 1 isolates, in 14 per cent of those inoculated with Type 2 isolates, and in 23 per cent of those inoculated with Type 3 isolates. Non-paralyzed monkeys usually showed poliomyelitis lesions at the lumbar levels of the spinal cord.

Cerebral neurovirulence was found in three Type 3 isolates, but only after injection of doses as high as 10 million TCD_{50}.

A slight increase of neurotropism has been observed particularly among isolates of the second and third week after ingestion of live virus vaccine, and not among those isolated before the second and after the third week (Table 6 see p 365). Hence, the slightly increased neurovirulence seems to be of transitory character

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DISCUSSION

CHAIRMAN GEAR The papers presented by Dr Gard and Dr Verlinde are now open for discussion

DR LANGMUIR. I would like to comment on Dr *Gard's opening remarks* He made two comments One, I am sure, was in humor, about the negative control and the experience in Sweden, and the other, I think, was seriously, namely, about the experience in Israel being a basis of concern, that the inactivated vaccine will be an inadequate type of public health control measure

The Israel experience, to my knowledge, has not been adequately reported, and as all of us are concerned about it it seems to me that we need to have far more data than I have seen before making any gross generalizations

I think it is proper, however, that the evidence for a substantial effect of inactivated vaccines should at least be mentioned at this meeting The evidence is more than merely a decline in the annual incidence rates, in a relatively small country such as Sweden We have observed similar declines in separate states over a number of years but the decline in the past four years has involved the whole North American Continent

But also far more important has been a radical change in the whole pattern of the behavior of paralytic disease in this country From a disease which was universal in all socio economic groups polio in the last three or four years in the United States has reverted to an infantile character, and to a concentration, to an extraordinary degree, in preschool uninoculated ethnic groups of various types

In the Detroit epidemic last year the difference in attack rates between Negro and white populations was 18 to 1, whereas in almost all large urban epidemics before 1955 attack rates by different types of socio-economic groups were amazingly similar

Now we obviously are watching very carefully for evidence of a break in the immunity level from an inactivated type of vaccine We do not expect the duration to be everlasting Many of us thought it might come rather soon As yet we have not found any evidence of a break, and it is for this reason that I discount the Israel experience as evidence of failure of the Salk type of vaccine until far more evidence has been put on the line than I have seen

DR PAYNE Concerning the Israel experience which Dr Langmuir has mentioned, the data we have is not complete, and, unfortunately, the analysis of the data is rather complex because of the different groups involved Some groups received two doses of vaccine by the subcutaneous route, others received two doses by the intradermal route plus a booster dose by the subcutaneous route, and others received no vaccine

The great difficulty in interpretation results from carrying out the initial vaccination by the intradermal route, and then in the following year those children were given a booster dose by the subcutaneous route, whereas the new vaccinees were only given two doses by the subcutaneous route

The age incidence of paralytic polio in Israel falls very sharply with rising age, so a difference between the incidence in triply vaccinated children who were mostly older and that in doubly or singly vaccinated children does not necessarily reflect protection

I think we will have to await the final details before this can be analyzed But I would like to stress that in Israel the vaccine was not administered according to the recommendations in force in this country—the intradermal route was used to a considerable extent The vaccine used *at that time was of the relatively lower potency* of the vaccines which were produced two or three years ago

The vaccines on the market now, I understand, have shown a steady rise in potency, particularly of the Type 1 component In Israel they have made great efforts to improve their vaccine especially the Type 1 component, and the serologic results suggest they have succeeded to a very large extent

TABLE 5. PARALYTIC INCIDENCE IN CYNOMOLGUS MONKEYS FOLLOWING INTRASPINAL INOCULATION OF ATTENUATED POLIOVIRUSES EXCRETED BY VACCINATED INDIVIDUALS THROUGH THE THIRD AND THE FOURTH INTERVERTEBRAL SPACE ABOVE THE ILIAC CRESTS

TYPE	DOSE (TCD_{50} LOG_{10})	3RD SPACE PARALYSIS	4TH SPACE PARALYSIS
1	5 6—6 5	0/8	3/8
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3	6 1—6 5	0/6	2/6

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Cerebral neurovirulence was found in three Type 3 isolates, but only after injection of doses as high as 10 million TCD_{50}

A slight increase of neurotropism has been observed particularly among isolates of the second and third week after ingestion of live virus vaccine, and not among those isolated before the second and after the third week (Table 6, see p 365). Hence, the slightly increased neurovirulence seems to be of transitory character.

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4 Sabin, A. B Brit M J *5123*, 663, 1959

4. THE USE OF ORALLY ADMINISTERED LIVE ATTENUATED POLIOVIRUSES AS A VACCINE IN A COMMUNITY SETTING: A CONTROLLED STUDY*

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Dr Barr *(presenting the paper)* This paper will be presented jointly with Dr Kleinman, the epidemiologist of the Department, and Dr Bauer, who will comment on laboratory findings

I would like to call attention first to the paper presented by Dr Dick, in which he brought out the problems in connection with oral vaccine

These were the same problems that the Minnesota *State Board of Health* faced after Dr. da Silva had given vaccine to a number of infants at the University of Minnesota We had done the laboratory work on that group, and suggestions for a further study were made

The Board of Health reactivated its Polio Advisory Committee, headed by Dr Gaylord Anderson, and reviewed the problems, including the calculated risk in using oral attenuated vaccine in a group of people

The policy of the Board of Health has always been to make complete information available to the people and to the members of the medical profession concerned with these types of studies

They recognized that there was a certain amount of calculated risk in feeding vaccine at this time, and they so stated, and they also stated that, if they did not proceed they would be more derelict in their duties by doing nothing, than they would by going ahead and doing a good job

The next point they raised was that every bit of information of value that could be pulled out of such a study must be pulled out, and the study must be so planned and designed that this information can be derived as completely as possible

Lastly, the individuals who were members of the study group should know about the entire picture and should be included only on a volunteer basis

I might say that this was not accomplished overnight, it took a little time Today we are still talking about the safety of this vaccine—its safety, as brought out by Dr Dick, both at the time it is administered and after it has passed through large numbers of individuals

We are talking about the question of the amount of antibody stimulation it gives, in other words, the protection that may be derived from it And we are talking about whether antibody titers actually represent protection or not

Obviously, this will not be completely determined until a large number of people so immunized are challenged with a virulent organism

We do not propose, in Minnesota, to go out and challenge those we fed in the villages with a virulent Type 1 virus

We should also add that the studies were made with the financial assistance and the biologics provided by the Lederle Laboratories, and with financial assistance from the Kenny Foundation

I also want to make it quite clear that the Department of Health set up the study and ran it, and in no way were the determinations influenced by the parties that were assisting financially

We feel that we have contributed in a small way to this total study, and that if such contribution is of value to the entire study group, then that is splendid If the vaccines we used, or other vaccines, turn out to be the ones that are

* Supported in part by grants from the Sister [illegible]

[illegible] Bureau/World Health Organization), Dr Kimball (Minnesota Department of Health), and Miss Cooney (Minnesota Department of Health)

DR BODIAN: I would like to congratulate Dr Gard on obtaining in human beings evidence which seems to confirm the experimental work of Dr. Howe in chimpanzees on the relationship of antibody level to excretion of virus.

It seems that we have to look at the antibody level and possibly to other factors inherent in so-called local immunity

I want to add also my interest in seeing the last two speakers add to the series of approximately one half dozen statements from speakers whom we have heard during the meeting, which have shown a less than complete genetic stability of these viruses

DR RHODES I fully realize that this Conference is not called to deal extensively with the inactivated vaccine, but I do wish to follow up a remark from Dr Langmuir I think this point will be of interest for the record, in that the results have not yet been published, but will be shortly.

In the city of Winnipeg Manitoba, which is right in the middle of Canada in the prairies there was a very severe epidemic of paralytic polio in 1958 The attack rates have been calculated for the non-vaccinated and the vaccinated

In all cases the vaccine used was the Salk type prepared at the Connaught Medical Research Laboratories, University of Toronto

I will deal with the ages of 6 to 14 The attack rate per 100,000 in the non-vaccinated was 177, in the triply vaccinated it was 2, giving a per cent decrease of 99 *The actual total figures of* cases were 23 and 1, respectively

This, I think, could be added to Dr Langmuir's comments

DR VERLINDE. I may add to the remarks of Dr. Bodian that in our experiments we certainly did not find a complete correlation between the antibody level and the susceptibility to poliovirus infection The correlation seemed to be complete as far as Type 2 was concerned, but certainly did not in the case of Type 3, where we found in some individuals considerable excretion of virus in the presence of a high level of antibody of 1024, and this was also true to a certain extent in the case of Type 1.

DR. GARD. I hope that my introductory remarks did not convey to the audience the impression that I deny that the inactivated virus would have any immunizing and protective effect I certainly do not do that.

What I have tried to say was that we know very little about what degree of protection we can achieve with inactivated virus, that is, protection against the massive exposure that takes place during widespread epidemics of a highly invasive strain of virus. And I do not think that thus far any country has any experience in this respect except Israel, and that experience, although a final analysis may not yet be possible, indicates that the protection was far from complete

To sum up, I still think that we have to find methods to improve our present procedures for immunization, particularly against Type 1

I would also add a comment on a statement that Dr Verlinde made He stated that, according to the observations in Holland, there apparently existed higher resistance of the alimentary tract in adults, as compared with children

Now, we had in our material 14 adults in which we did not detect antibody in pre immunization studies However, of those 14, when inoculated with inactivated virus, 4 reacted with a typical booster effect, and these 4 later proved to be resistant on feeding of live virus The remaining 10 individuals did not react with booster effects *on administration of inactivated* virus, and were later found just as susceptible to infection as the children were They excreted virus for an average period of 4 9 weeks, as compared with 5 2 weeks in children

I think that the classification of susceptibles on the basis of pre immunization tests alone, is a little uncertain, if sufficiently sensitive tests for detection of antibodies are not used

Thus, we could not find in our group any indication that susceptibility was determined by age alone It was entirely dependent upon previous exposure and, to a certain extent, upon the level of antibody at the time of feeding.

Fig 1a Passage History of SM Virus (Type 1)

Monkey Virulence Test						
Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Chick Embryo Tissue Culture	Times Plaqued in Monkey Kidney Cells
				S+M Pool (MK-TC) ↓ Mice I S 26 Pas ↓		
				10 pas →	14 pas →	No 1
				2 pas	5 alternations	
				1 pas		
7 2	0/29	6 5	2/23			
6 2	0/8	5 5	2/7			
5 2	0/8	4 5	2/7	Pool N90 (7 5)* →		No 2
4 2	0/8	3 5	0/7			
3 2	0/8	2 5	0/3			
				2 pas		
7 2	0/4	6 5	0/1	Pool 19 (7 5)* →		No 3
				2 pas		
7 9	0/4	7 2	0/4	Pool 21 (8 2)* →		No 4
				2 pas		
—	—	6 7	1/5	Pool 42 (7 7)*	1 pas	
5 4	0/10	4 7	0/5		Pool 45 (5 7)*	
6 9	0/10	6 2	0/10	1 pas		
—	—	5 2	3/10	Pool 100B-10-M3 (7 7)* (Seed virus)		
7 7	0/3	6 7	6/9	1 pas		
6 7	0/9	5 7	4+1?/8	Pool 7-1231-110 (7 7)*		
5 7	0/9	4 7	3+1?/10	Fed in capsules		

* Log TCD_{50} per ml

the most desirable, then that is good The basic purpose of all of us here, I am sure, is to determine the best ways in which we can protect the people of our city, our country, our world, against poliomyelitis

I shall now present the text of the paper we have prepared, which is as follows

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Efforts to develop attenuated living strains of poliovirus for use as immunizing agents against the disease poliomyelitis have been concentrated within the years of the last two decades The names most prominently associated with these efforts are those of Cox,[1] Koprowski,[2] and Sabin[3] There is strong immunologic justification for such attempts The best and most durable immunity obtainable against a type specific virus disease is the immunity conferred by an attack of the disease itself, whether such an attack is a full blown clinical syndrome or a mild unrecognized, undiagnosed subclinical infection It is also a fact that the best immunizing agents now available against virus diseases are the living but modified viruses of smallpox and yellow fever Veterinary medicine can add numerous similar examples. The aim then is to duplicate natural infection and its resulting immunity but to do so with living agents so modified or attenuated that they do not in themselves produce overt clinical disease

There are inherent difficulties in controlling the formalinization processes involved in the commercial production of inactivated virus vaccine.[4] There has also been the recent admission that the vaccines so produced and currently in use have been of low potency[5] These two facts, together with the encouraging results so far reported for the vaccines composed of living attenuated poliovirus, are additional compelling reasons for the continuance of research in the field of vaccination against poliomyelitis with such living virus agents.

Up until two years ago human trials with attenuated poliovirus vaccines were limited to very small groups and these often living in an institutional environment But since then, and especially in 1958, the scope of human trials has boldly increased until now thousands of individuals have been fed Mass trials are either complete, in progress, or are being planned for the immediate future in Latin America,[6] Africa,[7] Asia,[8] and Europe.[9] For example, as of 10 March 1959 more than 550,000 people had been fed at least one type of the strains used in this study. Of these, 250,000 had received all three types[9] This study adds a mere 550 to the grand total; it has, however, the virtue of having been completely controlled.

DESIGN, MATERIALS, AND METHODS

The attenuated strains used in this study were developed under the direction of Dr. Herald R Cox The Type 1 strain started as a mixture of the Sickle and Mahoney strains of Type 1 poliovirus. The Type 2 strain was adapted from an original MEF_1 Type 2 The Type 3 strain was developed from virus isolated from a case of nonparalytic poliomyelitis in a one year old child and was originally supplied by Dr. John Fox, of Tulane University

The passage histories and the related monkey test results for pathogenicity for the three types are shown diagramatically in Figures 1a, 1b, and 1c The growth and pathogenicity characteristics of these modified strains will be adequately described elsewhere[10]

The strains used in this study are essentially the same as those used by Martins da Silva and his colleagues in a study carried out about one year previously which included only newborns and infants under six months[11] The results of this first Minnesota study indicated that the Type 2 strain was the least effective antigen Because of this, it was decided, for the present study, to double the dose and to feed this strain first After the Type 2 feeding, Types 1 and 3 were fed in sequence at three week intervals The dosage administered was computed to be 4 8-4 9 logs for Type 1, 5 1 logs for Type 2, and 5 3 logs for Type 3 The vaccine was dispensed in hard gelatin capsules with the virus adsorbed on granular gelatin

The population chosen for this study consisted of married University of Minnesota students and their children These families occupied a village site, maintained by the University, in the so-called southeast section of Minneapolis about five minutes drive from the main campus in that city The quarters occupied by these families were duplex back to-back structures of either the quonset hut, metal barracks, or prefabricated

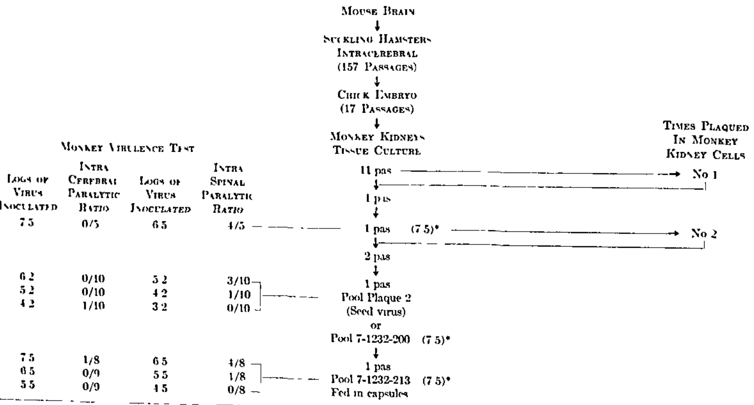
Fig. 1a Passage History of SM Virus (Type 1)

S+M Pool (MK-TC)
↓
Mice I S 26 Pas
↓

Monkey Virulence Test						
Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Chick Embryo Tissue Culture	Times Plaqued in Monkey Kidney Cells
				10 pas	→ 11 pas	→ No 1
				↓ 2 pas	5 alterations	
7 2	0/29	6 5	2/23	↓ 1 pas		
6 2	0/8	5 5	2/7	Pool N80 (7 5)*		→ No 2
5 2	0/8	4 5	2/7			
4 2	0/8	3 5	0/7	↓ 2 pas		
3 2	0/8	2 5	0/3			
7 2	0/4	6 5	0/4	Pool 19 (7 5)*		→ No 3
				↓ 2 pas		
7 0	0/4	7 2	0/4	Pool 21 (8 2)*		→ No 4
				↓ 2 pas		
—	—	6 7	1/5	Pool 12 (7 7)*	→ 1 pas	
5 4	0/10	4 7	0/5		Pool 45 (5 7)*	
6 9	0/10	6 2	0/10	↓ 1 pas		
—		5 2	3/10	Pool 100B-10-M3 (7 7)* (Seed virus)		
				↓ 1 pas		
7 7	0/3	6 7	6/9	Pool 7-1231-110 (7 7)*		
6 7	0/9	5 7	4+1?/8	Fed in capsules		
5 7	0/9	4 7	3+1?/10			

* Log TCD_{50} per ml

Fig 1B Passage History of MEF 1 Virus (Type 2)



* Log. TCD_{50} per ml.

Fig 1c Passage History of Fox Virus (Type 3)

Monkey Virulence Test: Logs of Virus Inoculated	Intra Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra Spinal Paralytic Ratio	Monkey Kidney Tissue Culture	Times Plaqued in Monkey Kidney Cells
				Human Stool (Fox P1149) ↓ Monkey Kidney Tissue Culture ↓	
				3 pas	→ No 1
				1 pas	
6 9	0/7	6 2	2/4	1 pas. (7 2)*	→ No 2
				2 pas	
7 7	1/14	7 0	1/8	1 pas (8 0)*	→ No 3
6 7	0/10	6 0	1/4	(same passage as 7 7 row)	
5 7	0/4	5 0	0/4	(same passage as 7 7 row)	
4 7	0/4	4 0	0/3	(same passage as 7 7 row)	
				1 pas.	
6 4	0/5	5 7	0/5	1 pas (6 7)*	
				1 pas	
7.2	0/14	6 5	0/4	1 pas (7 5)*	→ No 4
6 2	0/4	5 5	0/4	(same passage as 7.2 row)	
5 2	1/4	4 5	0/4	(same passage as 7.2 row)	
4 2	0/4	—	—		
				2 pas	
6 2	0/4	5 5	0/4	1 pas. Pool 9 (6 5)*	
7 5	0/5	6 5	0/5	1 pas Pool 7-1233-310 (7 5)*	
6 5	0/4	5 5	2?/5	(same passage as 7 5 row)	
		4 5	3/5	(same passage as 7 5 row)	
7 7	0/8	6 7	3/8	1 pas Pool 7-1233-318 (7 7)* Fed in capsules	
6 7	0/9	5 7	3/8	(same passage as 7 7 row above)	
5 7	0/9	4 7	0/8	(same passage as 7 7 row above)	

* Log. TCD_{50} per ml

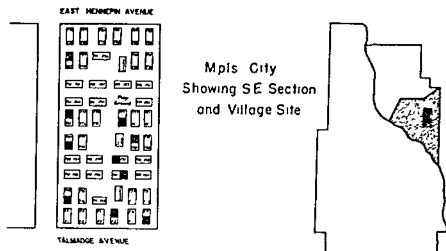


FIG 2 Site of oral poliomyelitis vaccine study—1958

wood types The layout of these dwellings, as well as the location of the site as a whole with respect to the rest of the city, is shown in Figure 2 *The village was surrounded by industry* and/or vacant space on the north, east, and south. Only to the west was there any residential area. The water supply and sewage facilities were those of the city itself.

The dwelling area, as can be seen in Figure 2, comprised six city blocks which were with one exception all adjacent, with each block filled with closely packed double dwelling units Not shown on the plan is a tier of blocks between Como and Talmadge Avenues, vacant except for the presence of the Village Students Union close to Como Avenue This building was the center of a busy communal life. It was a central meeting place, with facilities for the purchase of light meals and refreshments, reading materials, toys, etc In it were held such activities as governing council meetings, community suppers, a nursery school, dancing classes, and periodically, a well-baby clinic. There were also some recreation facilities and a room reserved for quiet study

In the village proper there was a great deal of visiting back and forth, daily coffee parties, and the frequent interchange of baby siters House-keeping *per se varied from excellent to very poor*, depending on the temperament of the individual housewife The entire environment was that of a closely knit, compact settlement that lived in cramped physical quarters on a hairline budget The intellectual caliber of the villagers, however, was above what one would normally expect from the occupants of an environment such as has been described.

The entire study was administered from an office located at the eastern edge of the village

Participation in the study project was on an entirely voluntary basis Nor was it required that an entire family unit participate as such Some families were represented by only a single participant; the largest participating group comprised a family of six individuals Previous experience with Salk vaccine to any degree was not a bar to participation. The final participating group represented about half of the total village population

TABLE 1A PARTICIPANTS AT BEGINNING OF STUDY, BY AGE AND VACCINAL STATUS (SALK) (149 FAMILIES)

AGE	NO VACCINE	ONE DOSE	TWO DOSES	THREE DOSES	TOTALS
Under 1	48	5	5	2	60
1	8	2	26	12	48
2	0	3	12	38	53
3	0	2	4	32	38
4	0	1	2	27	30
5	0	1	3	15	19
6	0	0	1	8	9
7	0	0	0	0	0
8	0	0	0	2	2
9	0	0	0	2	2
10	0	0	0	2	2
Fathers (Average age 27)	43	13	47	43	146
Mothers (Average age 26)	12	10	48	72	142
Totals	111	37	148	255	551

TABLE 1B SUMMARY OF PARTICIPANTS AT BEGINNING OF STUDY BY AGE GROUPS AND VACCINAL STATUS (SALK)

AGE GROUP	NO VACCINE	ONE DOSE	TWO DOSES	THREE DOSES	TOTALS
Children (Average age 2 4)	56	14	53	140	263
Fathers (Average age 27)	43	13	47	43	146
Mothers (Average age 26)	12	10	48	72	142
Totals	111	37	148	255	551

The characteristics of the participating population (at the beginning of the study) with respect to age and vaccinal status with Salk vaccine are shown in detail in Table 1a and, somewhat summarized, in Table 1b. On the basis of age, the population divides itself into two groups: a younger group of children mostly under the age of six, and an adult group composed of the mothers and fathers. The mean age for the children was 2 4 years, for the fathers 27 years, and for the mothers 26 years Almost half the population had had the recommended three doses of Salk vaccine The largest contributors to this group were the children over one year of age and the mothers About one-fifth of the participants had had no Salk vaccine at all The largest contributors to this group were the children under one year of age and the fathers There were relatively few (37) who had had only one dose of Salk vaccine and those with two doses were about evenly divided among children, fathers, and mothers In the main, then, it was the women and children who had received the complete Salk dosage and the men and infants who had had none All participants had to agree not to take any Salk vaccine for the duration of the study and, so far as is known, this agreement was not broken.

The location of and the population involved in the study were, of course, not completely typical of either a rural or an urban community Nevertheless, there were distinct operational advantages in working with such a group in such a location This community yielded a group of participants concentrated in space to an area of six city blocks The entire area was only a short distance from the laboratories of the Minnesota Health Department on the University of Minnesota campus The advantages in time and travel when it came to actually administering and supervising the details of the study are immediately obvious Further, it was felt that this group, more than any other because of training and outlook, could most readily appreciate the implications, both theoretical and actual, that were involved in such a study. How well they responded to all the implications, including even the odious task of collecting frequent stool specimens, will become apparent later

The design of the study was directed toward achieving three experimental conditions: first the creation of a control group and control period, second, *complete and continuous supervision* of all participants; third, freedom from bias in interpreting clinical information and laboratory results.

To establish a control group and a control period, at the very outset the participating population was divided into two groups on a purely random basis These groups were designated A and B The A group was the control, or placebo, group which received placebo capsules, while the B group received capsules containing the attenuated virus types. The component unit in each group was the family, not the individual. That is, no family contained both placebo-control and virus-fed individuals at the same time, it was entirely placebo or entirely virus fed. The distribution of these groups within the village is shown in Figure 2 In order that all participants might eventually receive the vaccine, the procedure was reversed for the two groups after the first round of feeding The sequence of this procedure appears in Table 2

Supervision was effected by means of a detailed system of record keeping, through the activities of two public health nurses who were concerned mostly with day-to-day mechanics, and through the maintenance of liaison with the individual families, both through the public health nurses and at times with the families' own physicians Recording of pertinent data was, in general, the responsibility of a specially employed secretary although the nurses themselves recorded family and individual histories as well as daily progress notes when such were indicated

Each family had its own data sheet, and each individual participant within a family had his own record sheet The family sheet contained identifying data for each participating member as well as a listing of the usual daily contacts for each member in the order of frequency with which these contacts were made In addition there was space for the nurse to note the general state of hygiene, familial and personal, in each case Records of Salk vaccine status, dates of feeding, dates of bleeding and stool specimen submission were kept on the family sheet The results of serum antibody titrations and virus isolation attempts on stool specimens were also recorded on the family form as these became available

TABLE 2. CHRONOLOGY

	GROUP A			GROUP B		
DAY*	BLOODS	STOOL SPECIMENS	FEEDINGS	BLOODS	STOOL SPECIMENS	FEEDINGS
1— 6	1st			1st		
3— 11		1st			1st	
8— 13			Placebo			Type 2
28— 36		2nd			2nd	
35— 41			Placebo			Type 1
49— 59		3rd			3rd	
57— 62			Placebo			Type 3
70— 79		4th			4th	
71— 76	2nd			2nd		
78— 83			Type 2			Placebo
91— 99		5th			5th	
96—102			Type 1			Placebo
112—121		6th			6th	
116—122			Type 3			Placebo
134—139	3rd			3rd		

* Day 1 is January 27, 1958.
Days for stool specimen receipt and feeding overlap but each family was not given capsules until their stool specimens were received.

The individual's record sheet was essentially a condensed medical history form listing such items as physician's name and address, previous poliomyelitis history, prior serious illnesses, operations or injuries, and any present disabilities *Pregnancy was noted and the due date of delivery* recorded The immunization history was obtained in some detail with the date of the last dose of any antigen noted as accurately as possible A substantial part of the space on the individual record was given over to daily notes, to the recording of symptoms, intercurrent illnesses and any other information that might be deemed important

A generalized public health nursing service was offered to all village residents, participating or not The public health nurses visited each participant's home to complete the family and individual record forms as far as she could These nurses assisted at bleeding clinics, distributed stool specimen containers, and either fed or supervised the feeding of the capsules. These activities alone brought her into frequent contact with the families In addition she was subject to call for consultation on any problem that a family wished to discuss with her Participants were encouraged to report illnesses or symptoms to the public health nurses

A letter was sent to the physician named by the participants informing him that the family was taking part in the study. The family physician was further informed that participants were instructed to use ordinary and usual medical facilities for any illness which occurred during the period of the study. In other words, they were urged to consult their own physician in case of illness. The physician was further invited to use the public health nursing service that had *been made available* in any manner which he thought convenient and beneficial to the patient and himself Where no family physician was named a similar letter was sent to the Students' Health Service of the University

Only one person in the project team knew the identity of the individuals who comprised the A. *placebo-control* group, and the *B* virus-fed group This identity was not divulged until the end of the study period The participants thus never knew until the end, whether and when they had received actual virus or placebo The project team, with the one exception noted, was in a like position. There could therefore be no

bias in the clinical interpretation of symptoms that were reported.

A set of three blood specimens collected at the intervals shown in Table 2 was obtained from each participant These were sent to the laboratory with a label that identified their origin and sequence by code number. All specimens in any one set were always tested on the same day But when these sets were actually tested, the original identifying labels had been replaced by a scrambled and random numbering system that made it impossible for the testing personnel to identify any one specimen as to its individual origin, its set origin, or its sequence within any one given set. The laboratory crew that received the specimens, separated the serum, and made the first dilution, was different from the crew that did the antibody titrations Indeed, the two crews worked in different places in the laboratory quarters and there was no overlapping of personnel

The schedule for bleeding, collection of stool specimens and feeding both placebo and vaccine is set out in detail in Table 2 The study got under way on 27 January 1958 and, except for the laboratory work, was completed 140 days later, in June 1958 This was well in advance of the usual Minnesota poliomyelitis season

Operations started off by first securing a blood *specimen and a stool specimen from each participant* These specimens had to be submitted before feeding About one week was consumed for each bleeding, each stool specimen collection and each feeding The days for stool specimen receipt and feeding overlapped, but no family was fed initially or serially until its entire complement of stool specimens had been received Reference to Table 2 will show that each subsequent feeding in a series was preceded by the collection of a stool specimen for a total of six such specimens The second blood specimen was collected between the 71st and 76th day, about the 14th day, on the average, after the third feeding The third and last blood specimen was collected between the 134th and 139th day, which was some 17 or 18 days, on the average, after the last of the second round of feedings

It would be well to accurately delineate here the control period and definitely identify the control population. The control period extended from day one through day 76, during which time the A group received placebo material while the B group was virus-fed. This point is delineated on Table 2 by a broken line. It is apparent, too, that the control population would number approximately half of the total population and that the placebo group's antibody picture would be represented by the results of the antibody titrations on their first two blood specimens Since after the control period the placebo group was in turn virus fed, the ultimate measure of the vaccine's effect with respect to serum antibody response would include the entire participating population and would be represented by the results of antibody titrations on the first and third blood specimens of everyone in both groups A and B

The capsules containing either virus or placebo material were delivered to the homes by the public health nurses Those who could swallow capsule size medication did so The others, infants and children, were spoon fed by mixing the capsule contents with any acceptable liquid The nurse was thus assured of the ingestion of the material for those at home during the time of her visit The capsules were left for those not at home, usually the father and sometimes a school child No attempt was made to relate the feeding to meal times

Certain contraindications to *feeding* were *agreed upon in advance Current* vomiting and/or diarrhea were obvious contraindications that demanded a delay in feeding until these symptoms had disappeared. Feeding was delayed for any illness that had a febrile episode until the person's temperature had stabilized itself at a normal range Open lesions of the oral cavity or the pharynx, whether surgical or due to disease, called for a delay in feeding until these lesions had healed over Only a single family had to be dropped from the study because of a continuing contraindication This was a family in whom the mother developed considerable dental trouble necessitating frequent extractions This situation became apparent after one virus feeding and the family thereafter was assigned placebos for the duration of the study

EVALUATION OF SYMPTOMS OCCURRING DURING THE CONTROL PERIOD

Recording and evaluating illnesses and symptoms that occurred during the study period was

of paramount importance if the presence or absence of "reaction" symptoms was to be established on a sound basis. During the preliminary phases of the study, it was learned that a good deal of illness could be expected in the village. This was the usual experience. The villagers themselves volunteered that there was always "some kind of cold or flu going around." Pediatricians who had villagers for patients in the past predicted the usual yearly outbreak of illnesses.

Table 3 is a list of illnesses classified under four heads as they occurred between the first and second, the second and third, and the third and fourth feedings. These time intervals cover the span of the control period. The upper respiratory infections group included such items as the coryzas, pharyngitis, tonsillitis, bronchitis, middle ear infections, etc. The gastro-intestinal group was composed of the fever, vomiting and diarrhea complex. The common communicable disease group was mostly chicken pox and mumps. The miscellaneous group was heterogeneous, containing entries as diverse as trauma and the allergies.

Table 3 was constructed before the real identities of the placebo-control and the virus-fed groups were known. It cannot, therefore, be used to compare the illnesses in the two groups. However, it does serve to show the pattern of illness in the village through the control period. That there was a pattern is self-evident. The predominant type of illness in February between the first two feedings was of the upper respiratory infection type. The predominant illness between the second and third feedings late in February and through the month of March was the gastro-intestinal disturbance. Remarkably enough, the percentage occurrences of these two types of illness through both the designated time intervals are almost precisely interchangeable.

The two sections of Table 4 (4a and 4b), one for adults and one for children, were constructed after the identities of the group A and group B participants became known. The table is arranged so that it is possible to compare the illnesses in the placebo-control group with the illnesses in the virus-fed group, week by week, through the control period. For this table the diagnosed communicable diseases were dropped from consideration as were other illnesses and complaints that were grossly remote from being considered as a possible "reaction." The upper respiratory and gastro-intestinal groups remain in the table. Fever alone, without any other symptoms or signs, has been added. The category "other" includes such symptoms as might possibly be due to the effect of virus on the central nervous system.

There was, of course, some variation in the relative frequency with which symptoms or illnesses occurred week by week in the two participating groups. But for those weeks when the reported incidence for any type of illness was at its peak, the comparative frequency was very close, if not identical. For example in adults note the comparative frequency of reports of upper respiratory disease for the week of 9 February and the comparative frequency of gastro-intestinal disturbance for the week of 17 March. Similarly, in children note the incidences of upper respiratory disease for the weeks of 9 and 24 February and the incidences of gastro-intestinal disturbances from 24 February on. There were only two adults with fever as the sole symptom, one in the placebo-control group and one in the virus-fed group. Among children there were ten such recordings, with the placebo-control group showing eight and the virus-fed group

Table 3. Types of Illness Occurring by Per Cent in Participants During the Control Period (First Half of Study)

	Between Feedings 1 and 2 February	Between Feedings 2 and 3 February—March	Between Feedings 3 and 4 March—April
Upper Respiratory Infections	65.0	18.8	44.8
Gastro-Intestinal Disturbances	18.9	69.2	29.8
Common Communicable Diseases	4.0	2.3	10.3
Miscellaneous	12.1	9.7	15.1

bias in the clinical interpretation of symptoms that were reported.

A set of three blood specimens collected at the intervals shown in Table 2 was obtained from each participant These were sent to the laboratory with a label that identified their origin and sequence by code number All specimens in any one set were always tested on the same day But when these sets were actually tested, the original identifying labels had been replaced by a scrambled and random numbering system that made it impossible for the testing personnel to identify any one specimen as to its individual origin, its set origin, or its sequence within any one given set The laboratory crew that received the specimens, separated the serum, and made the first dilution, was different from the crew that did the antibody titrations Indeed, the two crews worked in different places in the laboratory quarters and there was no overlapping of personnel

The schedule for bleeding, collection of stool specimens, and feeding both placebo and vaccine is set out in detail in Table 2 The study got under way on 27 January 1958 and, except for the laboratory work, was completed 140 days later, in June 1958 This was well in advance of the usual Minnesota poliomyelitis season

Operations started off by first securing a blood specimen and a stool specimen from each participant These specimens had to be submitted before feeding About one week was consumed for each bleeding, each stool specimen collection, and each feeding The days for stool specimen receipt and feeding overlapped, but no family was fed initially or serially until its entire complement of stool specimens had been received Reference to Table 2 will show that each subsequent feeding in a series was preceded by the collection of a stool specimen for a total of six such specimens. The second blood specimen was collected between the 71st and 76th day, about the 14th day, on the average, after the third feeding. The third and last blood specimen was collected between the 134th and 139th day, which was some 17 or 18 days, on the average, after the last of the second round of feedings

It would be well to accurately delineate here the control period and definitely identify the control population. The control period extended from day one through day 76, during which time the A group received placebo material while the B group was virus-fed. This point is delineated on Table 2 by a broken line. It is apparent, too, that the control population would number approximately half of the total population and that the placebo group's antibody picture would be represented by the results of the antibody titrations on their first two blood specimens Since after the control period the placebo group was in turn virus-fed, the ultimate measure of the vaccine's effect with respect to serum antibody response would include the entire participating population and would be represented by the results of antibody titrations on the first and third blood specimens of everyone in both groups A and B

The capsules containing either virus or placebo material were delivered to the homes by the public health nurses Those who could swallow capsule size medication did so The others, infants and children, were spoon fed by mixing the capsule contents with any acceptable liquid The nurse was thus assured of the ingestion of the material for those at home during the time of her visit The capsules were left for those not at home, usually the father and sometimes a school child No attempt was made to relate the feeding to meal times

Certain contraindications to feeding were agreed upon in advance Current vomiting and/or diarrhea were obvious contraindications that demanded a delay in feeding until these symptoms had disappeared Feeding was delayed for any illness that had a febrile episode until the person's temperature had stabilized itself at a normal range Open lesions of the oral cavity or the pharynx, whether surgical or due to disease, called for a delay in feeding until these lesions had healed over Only a single family had to be dropped from the study because of a continuing contraindication This was a family in whom the mother developed considerable dental trouble necessitating frequent extractions This situation became apparent after one virus feeding and the family thereafter was assigned placebos for the duration of the study

EVALUATION OF SYMPTOMS OCCURRING DURING THE CONTROL PERIOD

Recording and evaluating illnesses and symptoms that occurred during the study period was

two. The three adult individuals who showed "other" symptoms were all in the placebo-control group The two with headaches alone admitted that they frequently suffered from this complaint. The symptoms of headache, dizziness, and questionable stiff neck in the third adult appeared within 24 hours of feeding and disappeared overnight Among the virus-fed children there was one complaint of dizziness, one of headache, and one of soreness in the neck. None of these symptoms persisted One of the placebo-control children developed red "blotches" on the body. The exact nature of this rash was not determined

It appears, therefore, that over all there was no overwhelming preponderance of symptoms or symptom constellations limited to one or the other of the experimental groups The differences in the total occurrences of symptoms of all kinds noted below Table 4 are not statistically significant for either adults or children There is no reason, therefore, to believe that the virus was responsible for any type of symptom or any type of reaction pattern

In addition, it is interesting to note that the pattern of illness shown in Tables 3 and 4 was exactly the pattern of illness that was occurring in the city outside the village limits The participants themselves observed that the non-participants in the village were coming down with exactly similar illnesses Certainly it is no surprise to note a good deal of respiratory infection in February and March The gastro-intestinal disturbance was occurring not only in the village, but also in the city at large, and indeed over the entire state Reports of diarrhea epidemics were coming into the state health department from communities more than 100 and often 200 miles away from Minneapolis and from all corners of the state

Members of the project team examined seven families in whom this gastro intestinal disturbance was occurring Four of these were in the A group and three in the B group, although at the time the examiners did not know which group was virus fed They found the disease to be a definite clinical entity similar in nature in all the families examined regardless of group designation

LABORATORY METHODS

Preparation of TC tubes for antibody titrations HeLa cells were grown in milk dilution bottles which were seeded with 10^4 cells in 10 ml. of growth medium and incubated at 37°C for two days when 2 ml of human serum were added A replacement feeding of 5 ml of growth medium was made on the fourth and sixth days of incubation If cell growth had not followed the expected pattern, the feeding schedule was varied After a total of seven days of incubation, cells were removed from the bottle surface by trypsinization or scraping with a rubber "policeman" Cells from seven to ten bottles were pooled and a cell count made in a hemacytometer The cell suspension was diluted in growth medium to contain 10^3 cells per ml and dispensed in 0 5 ml quantities into five-inch tissue culture tubes The tubes were tightly stoppered with rubber stoppers and incubated in a stationary position in Seelye racks for three or four days. Growth medium was removed and the cell sheets in the tubes were rinsed three times with 1 0 ml of Hanks' BSS to remove all trace of human serum Maintenance medium * (0 9 ml.) was dispensed into each tube

Poliovirus pools were prepared with unmodified strains in quantities sufficient to use through the entire study Growth medium was removed from HeLa cell bottle cultures which had a population

*1 Lactalbumin Hydrolysate-Yeast Extract (LHYE)	
a Lactalbumin Hydrolysate	5 0 gms
b Glucose	4 5 gms
c Yeast Extract	1 0 gm
d 1% Phenol Red	1 6 ml
e Earl's BSS	1 liter
2 Growth Medium	
a LHYE	80 0 ml
b Human Serum	20 0 ml
c 5% $NaHCO_3$	2 0 ml
d Penicillin	100 units/ml
e Streptomycin	100 mcg /ml
3 Maintenance Medium	
a LHYE	93 0 ml
b Polio Antibody free calf serum	2 0 ml.
c 5% $NaHCO_3$	2 0 ml
d Penicillin	100 units/ml
e Streptomycin	100 mcg /ml
4 Diluent	
a Hanks' BSS	85 0 ml
b. 2% Yeast Extract	5 0 ml.
c 1 4% $NaHCO_3$	5 0 ml.
d 10% Glucose	2.5 ml
e Penicillin	100 units/ml
f Streptomycin	100 mcg./ml

TABLE 4A. OCCURRENCE OF SYMPTOMS BY CLINICAL TYPE AND BY EXPERIMENTAL GROUP DURING THE CONTROL PERIOD (FIRST HALF OF STUDY)

CHILDREN

Period	Respiratory		Gastro-Intestinal (Diarrhea)		Fever Alone		Other	
	Virus	Placebo	Virus	Placebo	Virus	Placebo	Virus[1]	Placebo[2]
*Feb 3—Feb 8	2	4	2	2		2		
" 9— " 15	10	10	2	2			1	1
" 16— " 23	8	4	4	1		3		
* " 24—Mar 1	14	14	6	7	1	2		
Mar 2— " 8	3		5	3		1	1	
" 9— " 16	2	2	8	7				
* " 17— " 22	5	3	19	12			1	
" 23— " 31	1	2	4	3	1			
Totals	45	39	50	37	2	8	3	1

Total in virus group, 132
Total in placebo group, 125
Total symptoms of all kinds in virus group, 100/132 (75.8%)
Total symptoms of all kinds in placebo group, 85/125 (68.0%)
* Denotes feeding dates
[1] One complaint of dizziness, one of headache, and one of soreness in neck region
[2] Red "blotches" on body

TABLE 4B. OCCURRENCE OF SYMPTOMS BY CLINICAL TYPE AND BY EXPERIMENTAL GROUP DURING THE CONTROL PERIOD (FIRST HALF OF STUDY)

ADULTS

Period	Respiratory		Gastro-Intestinal (Diarrhea)		Fever Alone		Other	
	Virus	Placebo	Virus	Placebo	Virus	Placebo	Virus	Placebo
*Feb 3—Feb 8	1	4	4	2				1[1]
" 9— " 15	14	15	3	2		1		
" 16— " 23	5	4	7					
* " 24—Mar 1	6	6	12	6	1			
Mar 2— " 8	4	2	7	1				
" 9— " 16	1	3	9	19				
* " 17— " 22	2	1	33	33				2[2]
" 23— " 31		3	12	6				
Totals	33	38	87	69	1	1		3

147

2.3%)
(78.2%)

[1] Headache, dizziness, stiff neck(?)
[2] Headache.

TABLE 5A ANTIBODY RESPONSES IN CHILDREN, VIRUS-FED

Post-feeding titers

Type 1

Pre-feeding titers	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	18	34	58	29	24	12	175	89.7%	70.3%
4	2(1)	3	6	11	8	5	35	85.7%	68.6%
16		1(1)	1	8		6	16	87.5%	37.5%
64		1(1)	2(2)	1	4	5	13	69.2%	38.5%
256			1(1)	1	4	3	9	33.3%	-
1024					1	6	7	-	-
Totals	20	39	68	50	41	37	255		

Type 2

Pre-feeding titers	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	13	12	27	23	20	9	104	87.5%	76.0%
4	6(5)	8	13	8	9	16	60	76.7%	55.0%
16		3(3)	3	7	8	18	39	84.6%	66.7%
64	1(1)		2(1)	6	7	8	24	62.5%	33.3%
256		1(1)		1	3	12	17	70.6%	-
1024					1	10	11	-	-
Totals	20	24	45	45	48	73	255		

Type 3

Pre-feeding titers	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	30	32	42	48	38	17	207	85.5%	70.0%
4	1(1)		6	5	5	2	19	94.7%	63.2%
16			1	5	4		10	90.0%	40.0%
64		1	3(1)	2	2		8	25.0%	0.0%
256		1		2	2	2	7	28.6%	-
1024				1		3	4	-	-
Totals	31	34	52	63	51	24	255		

Figures in parentheses denote infants.

of approximately 10^7 cells per bottle. Cell sheets were rinsed three times with 10 ml. of Hanks' BSS, after which 10 ml. of maintenance medium was added. Bottles were inoculated with 1 ml of poliovirus Type 1 (Mahoney), Type 2 (MEF_1) or Type 3 (Saukett) diluted 10^{-1}. Bottles incubated at 37°C. were observed microscopically daily for cytopathogenicity. When 75 to 100 per cent of the cells were destroyed the bottles were placed at −20°C. Pools of each type were made and were centrifuged (10 min., 2000 r.p.m., No. 2 horizontal) to remove cell debris. Each pool was dispensed into all glass ampules (0.3-0.5 ml. per ampule), sealed and stored at −70°C. Titrations on each pool were carried out in half-log dilutions to determine the $TCID_{50}$. Two or three ampules for each type pool were titrated. The $TCID_{50}$ was calculated by the method of Reed and Muench.

Serum specimens were stored at −20°C. Specimens collected prior to all vaccine and placebo feeding and those collected at the end of the control period (when group B had received vaccine and group A had not) were held until the third blood specimens, collected three weeks after the entire group had been fed, at which time all three specimens from each individual were tested in the same test run.

Neutralizing antibody test procedure. Five 4-fold serial dilutions (1:4 through 1:1024) of each serum specimen were prepared and dispensed in 0.15 ml. amounts into three rows of tubes. Each type of virus pool diluted to contain 100 $TCID_{50}$ per 0.1 ml. was added to one row of serum dilution tubes in 0.15 ml. amounts. Each serum-virus mixture (0.2 ml.) was inoculated into one TC tube after standing one hour at room temperature. Inoculated tubes were incubated at 37°C. for four days.

Controls. A control serum specimen previously titrated and known to contain neutralizing antibodies for each type of poliovirus was retitrated in each test run. Also with each test run TC tubes were inoculated with each type of virus pool diluted to contain 100, 10, and 1 $TCID_{50}$ to determine the precise quantity of virus used. An antibody run was not considered satisfactory unless the control virus titration showed the presence of at least 30 $TCID_{50}$ of virus. In most antibody runs, 100-300 $TCID_{50}$ of each virus had been present. Serum controls were not used routinely after 118 serum specimens proved to have no toxic effect on cells in a 1:4 dilution.

Reading. Inoculated tubes were examined microscopically after four days of incubation. Cytopathogenic effect was graded as approximately 25, 50, 75, or 100 per cent cell destruction. The neutralization end point was expressed as the reciprocal of the highest dilution of serum which completely neutralized the virus test dose.

RESULTS

The results of the antibody titrations are shown in Tables 5a and 5b for children and in Tables 6a and 6b, for adults. These tables combine the correlation square type of presentation with the percentage of positive responses, both gross and by 16-fold or greater rise displayed alongside each square.

In Table 5a, which deals with the virus-fed children, it can be seen that for each type the concentration of recorded results in the cells above the square's diagonal indicates that over all there was a marked tendency towards positive responses. In those who began the study with titers of less than 4, there was a change in a positive direction to any titer in 89.7 per cent of the cases for Type 1, in 87.5 per cent of the cases for Type 2, and 85.5 per cent of the cases for Type 3. Again beginning with those whose titers were less than 4 pre-feeding and counting only cases with a 16-fold or a greater rise in titer, it is seen that such a response was elicited in 70.3 per cent in Type 1, 76.0 per cent for Type 2, and 70.0 per cent for Type 3. The positive changes in titer for those who initially had titers of 4 or more might be considered "booster" effects. The extent of these at the various titer levels may be appreciated by consulting the tables.

The results for the placebo control children, as is evident in Table 5b, are markedly different. Here, the cells with recorded results tend to hug the square's diagonal and the percentages of positive responses, however measured, are either zero or of low magnitude. The general picture is that of no change in the post feeding status as compared to the pre-feeding status.

Certain of the cells below the diagonals show figures in parentheses. These represent the infant population of that particular cell. At first

TABLE 6A. ANTIBODY RESPONSES IN ADULTS, VIRUS-FED

Post-feeding titers

Pre-feeding titers	Type 1							Positive	Responses
	< 4	4	16	64	256	1024	Totals	Gross	16x or >
< 4	39	20	7	3		1	70	44.3%	15.7%
4	4	12	14	4	1		35	54.3%	14.3%
16		8	21	16	5		50	42.0%	10.0%
64		2	9	24	19	3	57	38.6%	5.3%
256			3	13	18	13	47	27.7%	-
1024				2	12	9	23	-	-
Totals	43	42	54	62	55	26	282		

Pre-feeding titers	Type 2							Positive	Responses
	< 4	4	16	64	256	1024	Totals	Gross	16x or >
< 4	26	19	10	1	1		57	54.4%	21.1%
4	2	13	5	8	2		30	50.0%	33.3%
16	1	8	21	11	11	3	55	45.5%	25.5%
64		3	14	15	14	8	54	40.7%	14.8%
256	1		3	13	22	11	50	22.0%	-
1024				4	14	18	36	-	-
Totals	30	43	53	52	64	40	282		

Pre-feeding titers	Type 3							Positive	Responses
	< 4	4	16	64	256	1024	Totals	Gross	16x or >
< 4	68	21	16	6	3		114	40.4%	21.9%
4	6	10	11	4	1		32	50.0%	15.6%
16		10	14	7	4		35	31.4%	11.4%
64		1	12	24	11	5	53	30.2%	9.4%
256			2	10	13	9	34	26.5%	-
1024				1	6	6	13	-	-
Totals	74	42	55	52	38	20	281		

TABLE 5B ANTIBODY RESPONSES IN CHILDREN, PLACEBO-CONTROL

Post-feeding titers

Type 1

	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	80	2(1)*	1	1	1*		85	5.9%	3.5%
4	11	5	2				18	11.1%	0.0%
16		2	5	1			8	12.5%	0.0%
64		1		2	1		4	25.0%	0.0%
256				3		1	4	25.0%	-
1024						3	3	-	-
Totals	91	10	8	7	2	4	122		

Type 2

	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	44	6(1)*	1		1*		52	15.4%	3.8%
4	14	11	1				26	3.8%	0.0%
16	1	6	11	3	1		22	18.2%	4.5%
64			4	8			12	0.0%	0.0%
256			1	2	3	1	7	14.3%	-
1024					2	1	3	-	-
Totals	59	23	18	13	7	2	122		

Type 3

	< 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	103	3		1			107	3.7%	0.9%
4	6						6	0.0%	0.0%
16	1	1	2				4	0.0%	0.0%
64		1					1	0.0%	0.0%
256			1		1		2	0.0%	-
1024					2		2	-	-
Totals	110	5	3	1	3	0	122		

Figures in parentheses denote infants
*Denotes virus isolation

glance, this might suggest a lack of antigenicity on the part of the vaccine for infants However, it should be remembered that in infants two forces are at work to determine the antibody level There is first the declining titer of the antibodies passively acquired by the infant from the mother Then there is the possible antigenic effect of the vaccine on the infant itself The resultant might very well be a "holding" action in which the attrition of maternally transmitted antibodies between the time the first and the last blood specimens were drawn (134 days) is masked, and in a sense overcome by the introduction of new vaccine-induced antibodies

It can be noted that there were some in the placebo-control children's group who did show a bettering of titer In four of these, homologous virus was recovered from the stool, indicating immunization by interfamilial spread from the virus-fed group Most of the other rises in titer in this class of placebo-control children were only 4 fold and could be considered within the limits of usual laboratory procedure variability Still there are four instances left where it is not an improbable conjecture that the immunization was effected by interfamilial spread of virus, even though such virus was not recovered from the stool of the beneficiary

The most that can be said for the analogous data for adults, as shown in Tables 6a and 6b, is that there appears to be a trend toward positive responses This trend is modest, but it is there Type 2 virus was recovered from two individuals in the placebo-control adult group, but there was no effect noted on the base-line antibody titer in these individuals

Another way to appreciate the effect of the vaccine on the participants is to note the change, or lack of change, that occurred with respect to the antibody profiles of the different groups. The antibody profiles are line graphs drawn by plotting on the ordinates the percentage of the population with antibody titers at various levels from less than 4 to 1024 as measured off on the abscissae These profile graphs are shown in Figures 3a and 3b The "before" and "after" profiles in the placebo-control group both for adults and children practically superimpose themselves one on the other In the virus-fed children there is evident a marked change in the contour of the "after" profile, a change indicative of a move to greater percentages with higher titers. The contour change for the virus-fed adults is not so marked, although it is quite obvious that for each type there are less adults left at the less than 4 level after virus feeding

The data presented so far represent the results obtained irrespective of prior experience with the Salk vaccine Even though the findings are available for each poliovirus type classified into the ultimate categories of fathers, mothers, children, and infants, each on the basis of prior Salk vaccine inoculations, no attempt will be made here to draw on analysis so fine. It can be appreciated that some of these ultimate categories turn out to be ridiculously small

However, because of the interesting implications, a summation of a selected set of such data will be shown These data are concerned with the degree of response to the orally administered attenuated poliovirus in relation to prior experience with Salk vaccine in those adults and children who entered the study with titers of less than 4 to any type of poliovirus These data are contained in Tables 6a and 6b. It is again emphasized that these tables include only those whose titers for any type were less than 4 before virus feeding For Tables 7a and 7b Salk vaccine experience has been divided into two classes one in which the experience was limited to no vaccine or to only one dose, and a second class in which the experience consisted of two or more doses of the Salk vaccine On the basis of past experience with the Salk vaccine, this appears to be a reasonable class division The tables in question contain the antibody titer results after virus feeding, arranged so that the titer arrays can be compared within the two above mentioned Salk vaccine experience classes for each poliovirus type and separately for adults and children The comparative conversion rates both gross and by 16 fold or greater increment are also entered The probability that the difference in response in the two classes was purely fortuitous, as determined by Chi square computations, is indicated by the p values opposite each couplet of titer sequences

It appears that everywhere, except for Type 1 in adults, the response to attenuated poliovirus was better in those who had had the most experience with the Salk vaccine It can be inferred then that the Salk vaccine has a "priming" effect

TABLE 6B. ANTIBODY RESPONSES IN ADULTS, PLACEBO-CONTROL

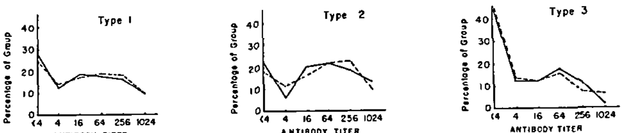
Post-feeding titers

Pre-feeding titers	Type 1 < 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	32	4					36	11.1%	0.0%
4	1	9	5	1			16	37.5%	6.3%
16	1	5	13	5	2		26	26.9%	7.7%
64		1	5	14	5		25	20.0%	0.0%
256			1	4	10	7	22	31.8%	-
1024				2	5	5	12	-	-
Totals	34	19	24	26	22	12	137		

Pre-feeding titers	Type 2 < 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	23	4	1				28	17.9%	3.6%
4	1	5	1		1		8	25.0%	12.5%
16		6	8 1*	12	1		27	48.1%	3.7%
64		1	8	8 1*	11	1	29	41.4%	3.4%
256			1	8	14	3	26	11.5%	-
1024			2	2	5	9	18	-	-
Totals	24	16	21	30	32	13	136		

Pre-feeding titers	Type 3 < 4	4	16	64	256	1024	Totals	Positive Gross	Responses 16x or >
< 4	54	7					61	11.5%	0.0%
4	7	6	2	2			17	23.5%	11.8%
16	1	3	10	2			16	12.5%	0.0%
64		1	4	11	6	2	24	33.3%	8.3%
256		1		6	4	4	15	26.7%	-
1024						3	3	-	-
Totals	62	18	16	21	10	9	136		

*Denotes Virus isolation.



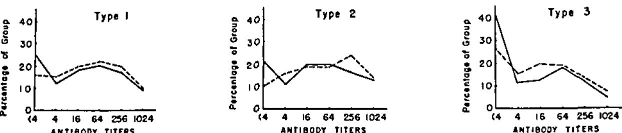
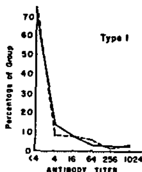
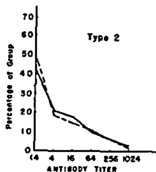
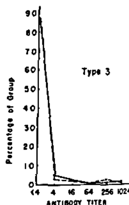


FIG 3B Antibody profiles—adults

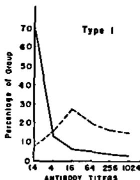
PLACEBO-CONTROL

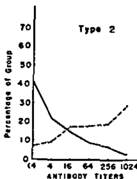


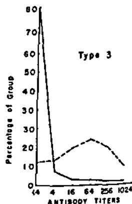




VIRUS-FED







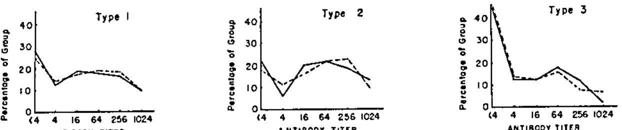
——— Before feeding
– – – After feeding

Fig 34 Antibody profiles—children

on the response to the attenuated poliovirus vaccine in those cases where the Salk vaccine either failed in the first place to elicit an antibody response or where the Salk induced titers were not maintained. Or, it may be postulated that the Salk vaccine did result in antibodies but at a level too low to be detected by the laboratory methods being used and that the subsequent response to the attenuated poliovirus vaccine was in truth an anamnestic one

Virus isolation procedures have now been completed on all stool specimens collected during the course of the study. Future publications will use the results obtained to consider the problem of interfamilial spread of the fed virus Likewise, the effect of the recovered virus on monkeys will be reported But, it is hard to resist presenting here and now the data which so dramatically demonstrate virus isolation rates as a function of antibody status and Salk vaccine experience These data are set out in Table 8 This table consists of two main sections, one of which contains the infants, children, and adults with antibody titers of less than 4 to the various poliovirus types, the other is similarly set up, except that it contains those individuals with titers of 4 or more to the various poliovirus types Each main section is then subdivided into the two classes of Salk vaccine experience as previously described The virus isolation rates are expressed as fractions, with the numerators as the actual number of isolations of homologous virus and the denominators as the number of stool specimens examined On the basis of the data in Table 8, three inferences seem justified

1 All persons with antibody titers of less than



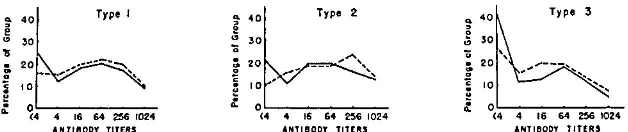


FIG 3B Antibody profiles—adults.

TABLE 7A EFFECT OF PREVIOUS SALK VACCINE EXPERIENCE ON ANTIBODY RESPONSE TO ORAL FEEDING WITH ATTENUATED POLIOVIRUSES

CHILDREN

No Doses Salk Vaccine	Total	Titer After Feeding* < 4	4	16	64	256	1024	Per cent Conversions	Per cent Conversions 16x or >	P
				Type 1						
0—1	51	7	10	23	10	0	1	86 3	66 7	< 01
2 or more	124	11	24	35	19	24	11	91 1	71 8	
				Type 2						
0—1	46	10	7	13	8	5	3	78 3	63 0	05
2 or more	58	3	5	14	15	15	6	94 8	86 2	
				Type 3						
0—1	54	15	10	12	11	4	2	72 2	53 7	< 01
2 or more	153	15	22	30	37	34	15	90 2	75 8	

TABLE 7B EFFECT OF PREVIOUS SALK VACCINE EXPERIENCE ON ANTIBODY RESPONSE TO ORAL FEEDING WITH ATTENUATED POLIOVIRUSES

ADULTS

No Doses Salk Vaccine	Total	Titer After Feeding* < 4	4	16	64	256	1024	Per cent Conversions	Per cent Conversions 16x or >	P
				Type 1						
0—1	29	13	11	3	1	0	1	55 2	17 2	> 25
2 or more	41	26	9	4	2	0	0	36 6	14 6	
				Type 2						
0—1	32	20	8	4	0	0	0	37 5	12 5	< 02
2 or more	25	6	11	6	1	1	0	76 0	32 0	
				Type 3						
0—1	39	30	4	1	1	3	0	23 1	12 8	< 02
2 or more	65	38	17	15	5	0	0	56 9	30 8	

*Pre-feeding titer in all cases < 4

4 have no local gut protection regardless of prior Salk vaccine experience

2 Adults who have antibody titers of 4 or greater and who have had no Salk vaccine or only one dose do show gut immunity to infection with homologous attenuated virus. Of necessity, both the local "tissue" and the "serologic" immunity in this group must have been acquired naturally through previous infection with "wild" poliovirus The almost completely negative stool results in this group are most striking

3. Infants with antibody titers of 4 or more do not show protection against intestinal infection possibly because the circulating antibodies which

TABLE 8 VIRUS ISOLATION RATES FROM STOOL SPECIMENS AS A FUNCTION OF ANTIBODY STATUS AND SALK VACCINE EXPERIENCE

Salk Vaccine Experience		Initial Titer <4		Initial Titer 4 or >	
		0—1 Dose	2 or More Doses	0—1 Dose	2 or More Doses
Adults	Type 1	12/29	13/41	0/46	8/166
	" 2	12/32	3/25	1/43	13/182
	" 3	8/39	15/65	0/36	3/141
Children	Type 1	14/16	86/119	0/1	28/65
	" 2	8/16	35/53	0/1	47/131
	" 3	7/17	72/146	0/0	3/38
Infants	Type 1	28/35	4/5	10/11	3/3
	" 2	22/30	3/5	12/16	1/3
	" 3	12/37	1/7	5/9	0/1

they passively receive across the placenta are of the same nature as Salk vaccine induced antibodies which do not, as is now well established, afford any protection against gut infection with virulent polioviruses As is evidenced here this same lack of local gut protection extends to attenuated polioviruses as well

CONCLUSIONS AND DISCUSSION

The orally-administered attenuated polioviruses used as vaccine in this study are clearly effective antigens for children This effectiveness obviously extends to all three types When measured by a crude bettering of an original antibody titer of less than 4, these viruses were effective in inducing such a change in from approximately 85 to 90 per cent of the participants When measured by the stricter and, from the laboratory standpoint, the more significant criterion of at least a 16-fold rise in titer, the effectiveness was still apparent in approximately 70 per cent of the participants

The vaccine was less effective in adults True enough, a trend is suggested in that the virus-fed group always had better conversion rates than did the placebo control group When the upward trend of antibody titers in adults is measured for statistical significance on the basis of 16 fold increments, the differences in response between the placebo-control group and the virus-fed group are actually found to be statistically significant for all three types While these differences are not so great as those that occur in the case of children, they are not accidental

TABLE 7A EFFECT OF PREVIOUS SALK VACCINE EXPERIENCE ON ANTIBODY RESPONSE TO ORAL FEEDING WITH ATTENUATED POLIOVIRUSES

CHILDREN

No Doses Salk Vaccine	Total	Titer After Feeding*						Per cent Conversions	Per cent Conversions 16x or >	P
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				Type 1						
0—1	51	7	10	23	10	0	1	86 3	66 7	< 01
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2 or more	58	3	5	14	15	15	6	94 8	86 2	
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2 or more	153	15	22	30	37	34	15	90 2	75 8	

TABLE 7B EFFECT OF PREVIOUS SALK VACCINE EXPERIENCE ON ANTIBODY RESPONSE TO ORAL FEEDING WITH ATTENUATED POLIOVIRUSES

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No Doses Salk Vaccine	Total	Titer After Feeding*						Per cent Conversions	Per cent Conversions 16x or >	P
		< 4	4	16	64	256	1024			
				Type 1						
0—1	29	13	11	3	1	0	1	55 2	17 2	> .25
2 or more	41	26	9	4	2	0	0	36 6	14 6	
				Type 2						
0—1	32	20	8	4	0	0	0	37 5	12 5	< 02
2 or more	25	6	11	6	1	1	0	76 0	32 0	
				Type 3						
0—1	39	30	4	1	1	3	0	23 1	12 8	< 02
2 or more	65	38	17	15	5	0	0	56 9	30 8	

*Pre feeding titer in all cases < 4

4 have no local gut protection regardless of prior Salk vaccine experience.

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		0—1 DOSE	2 OR MORE DOSES	0—1 DOSE	2 OR MORE DOSES
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	" 2	12/32	3/25	1/43	13/182
	" 3	8/30	15/65	0/36	3/141
Children	Type 1	14/16	86/119	0/1	28/65
	" 2	8/16	35/53	0/1	47/131
	" 3	7/17	72/146	0/0	3/38
Infants	Type 1	28/35	4/5	10/11	3/3
	" 2	22/30	3/5	12/16	1/3
	" 3	12/37	1/7	5/9	0/1

they passively receive across the placenta are of the same nature as Salk vaccine induced antibodies which do not, as is now well established afford any protection against gut infection with virulent polioviruses As is evidenced here, this same lack of local gut protection extends to attenuated polioviruses as well

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Attenuated viruses used in this study act like naturally occurring viruses in every respect save one They do not produce clinical disease. They do set up an intestinal infection, as is evidenced by the recovery of virus from the stool after an appropriate interval They do stimulate the production of circulating antibodies, as is evidenced by the antibody titrations Naturally occurring antibodies prevent the establishment of *an intestinal infection, serum antibodies* similar to those induced by Salk vaccination do not prevent such an infection Finally, the attenuated viruses appear to effect an anamnestic response in those who, at the moment, show no serologic evidence of having been successfully stimulated by the previous use of the formalinized vaccine

Definitive results were demonstrated in this study in spite of the fact that a substantial portion of the participants had received Salk vaccine This is important, as it indicates that the presence of a Salk vaccinated population should not deter further studies of this nature in this country In this connection it is interesting to record that of 140 children who entered the study with a history of three Salk vaccine inoculations, 57 per cent had titers of less than 4 to Type 1 poliovirus, 20 per cent had the same lack of antibody titers to Type 2 poliovirus, and 77 per cent had titers of less than 4 to Type 3 poliovirus

There is no good reason now to doubt that the administration of these living strains of poliovirus is a safe procedure Safety can be implied on the basis of testing in laboratory animals and *this is the method that must of necessity be used* when starting with any attenuated strain *de novo* The greatest confidence in the safety of these strains, however, comes with the knowledge that up to the present over a million doses of these viruses, including all types in completed or uncompleted sequences, have been consumed without report of any confirmed adverse effect

The question *of a return to* virulence is always being raised Specifically, it is asked, will passage through the human gut, once or serially, increase the neurotropism of these strains to the point of an actual return to virulence for humans? Some evidence exists to show that there is an increased neurotropism after one human gut passage, especially for Type 1, when compared to the neurotropic activity of the original virus used as vaccine There is also evidence that this neurotropism does not increase after a second passage; in fact it appears to diminish [11] This failure of neurotropism to persist after serial passages has been noted for attenuated strains other than those used in this study [12] Sabin has shown that some strains of virus shown to be neurotropic for monkeys by intraspinal injection are not neurotropic for chimpanzees He suggests that strains of this character could be safely fed to humans.[12] The team associated with this study feels that the occasional demonstration of neurotropism in monkeys following intracerebral or intraspinal injection of relatively large amounts of virus does not by itself constitute sufficient contraindication to the use of these strains in humans The neurotropic activity of the strains recovered from stool specimens submitted during the course of this project are now under study and will be reported in detail later

The investigators connected with this study realize that they have some unsolved problems on their hands The lack of responsiveness in adults is troublesome It has been suggested that this may be a matter of dosage It has also been suggested that gastric acidity may be an important factor since there is so marked a difference between the gastric acidities of adults and children Both of these suggestions are under trial now *Also* under trial is *the feasibility of immunizing* against all three types of poliomyelitis simultaneously with a single feeding of a trivalent preparation The durability of the antibodies *induced by the use of this* vaccine is not yet known It is hoped, with good reason, that they will be durable This is a question that time and follow-up alone can answer

Further, the investigators feel that the true effectiveness of an attenuated poliovirus vaccine, as well as any other type of vaccine, can be gauged only by its behavior in the face of an

actual challenge by the disease itself. Ideally, the quickest way to decide this question would be by a placebo-controlled study in an area where the natural incidence of poliomyelitis would be great enough to yield sufficient cases for statistical analysis. Better yet would be such a study during an epidemic situation. These strains have been used twice in association with epidemics of poliomyelitis, once in a Type 1 outbreak in Colombia[6] and again in a Type 2 outbreak in Nicaragua.[13] However, there were no control populations and the administration of the vaccine in each case was begun after the epidemic peak.

The *immunologic advantages* of a vaccine consisting of living attenuated poliovirus have been mentioned. The economic and administrative advantages are of equivalent importance. The simpler manufacturing process, the lesser costs involved, the ease of oral administration, the ability to eliminate the need for syringes and needles all make such a product ideal for use in those areas of the world where funds, medical equipment, and medical personnel are at a premium.

The results of this study are sufficiently encouraging to lead to the hope that the replacement of virulent polioviruses by attenuated polioviruses will effect the same degree of eradication for poliomyelitis as has been achieved for smallpox by replacing the variola virus with the vaccinia virus.

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DR. BARR: May I now introduce Dr Kleinman, who will comment further on this study

DR. KLEINMAN: You have just heard the detailed description of our 1958 study in Minnesota I should like, at this point, to mention some of the items that we believe to be of the most importance and of greatest interest in this entire study

In addition, I shall present certain data, not appearing in the manuscript, in connection with the recovery of a fed virus and its spread in the community which we studied, as well as some laboratory information on the persistence of the antibodies which we originally stimulated or induced

The population covered in the study consisted of married students of the University of Minnesota who were living a somewhat Bohemian and financially difficult existence in a village erected for their use close to the University This village comprised an area of about six city blocks The houses were duplex, back-to-back, single-story structures of the barracks type—either prefabricated, quonset huts, or similar types of structures

These people were highly intelligent, very cooperative, and very friendly, they visited among one another quite often There was frequent exchange of baby sitters, and there was also the opportunity for community contact, on a larger scale, in a students' union which operated on the grounds.

So far as age is concerned, two large age groups were represented One consisted of the fathers and the mothers, whose age ranged between 26 and 27 years, and the other consisted of children aged all the way from infancy up to six years, with a mean age of about 2 to 2½ years.

As regards their prior experience with the Salk vaccine, I can generalize by saying that the most Salk experience was had by the children and the mothers, and the least by the fathers and the infants

I should like now to go directly into a consideration of the design of this study, and in this design our objective was to create three experimental situations or conditions. First of all, we wished to construct a control group and a control period Secondly, we wished to be assured that there would be complete and continuous supervision of all participants. And third, we wished to work in an atmosphere that was as free from bias as possible, in interpreting the clinical information and the laboratory results

For the creation of a control group and a control period, at the very outset, in a random way, we divided the participating population into one group which was to receive a placebo preparation and another group which was to receive the actual attenuated viruses.

The unit within the experimental group was not the individual but the family. That is, no single family contained some individuals who received virus and some who received placebo; it was either all placebo or all viruses.

Table 2* shows the nature of the control group and the extent of the control period, and indicates also the entire plan of operation for the study, extending over 139 days, with day 1 on 27 January 1958

It can be seen that in the group under the heading "Feedings" we scheduled the administration of three placebos This is the placebo group, often designated by us as Group A

Also listed under "Feedings" is the group destined to receive the attenuated viruses, in the following order Type 2, Type 1, Type 3

The point at which the broken line is indicated in this table is the end of the control period and for all intents and purposes we could have stopped our work at that point But because we had promised the villagers that everybody would ultimately get the vaccine, we continued on; however, in the second half of the study we reversed the procedure, so that those who were originally the control group now received the vaccine in the same order, and those who had first received the vaccine now got three successive doses of placebo

As can be seen, the first thing we did was to draw a base-line blood This was the blood which would depict the antibody picture before the onset of the study. The next thing was the collection of a stool specimen in each group

And so the study proceeded Each feeding was preceded by the collection of a stool specimen A second blood specimen was collected after the first series of three feedings had been completed and a third blood specimen was col

* See p 377.

lected again after the entire village had been fed This, then, is the control period. This is the control group

It can be understood, then, that the control group in numbers is about one-half of the total group, and that the antibody picture of their response to placebo would be represented by the titrations on the first and second blood specimens

On the other hand, since ultimately everyone was fed the virus, the virus-fed group is the entire group, and the resulting antibody picture of those who received virus would be the results of the titrations done on the first blood and on the last blood

There was consumed a period of about one week, usually, in carrying out each one of the steps, although I can assure you that nobody received a feeding of either placebo or attenuated virus until he had anted up with the specimen neatly packaged

The final number of participants whose blood was titrated came to 537 Multiplying that by 3 it can be seen that we had to deal with some 1,600 blood specimens Inasmuch as there were 6 stool specimens collected, we ultimately had to deal with some 3 200 or more stool specimens

This, then, was how we established our control population and our control group

Insofar as supervision is concerned the back bone, the core, of our supervision lay in the institution of a complete, generalized public health nursing service for this village for the duration of the study This service was offered not only to participants, but also to non-participants While these girls were primarily concerned with the polio study, they were very carefully advised to act the role of a generalized public health nurse

The participants were urged to go to them for counsel, for advice, and for reporting

The stool containers were delivered by the nurses The feedings were either given by the nurses or supervised by them And if you count things up you will find that for each participant there were at least two dozen opportunities for scheduled or enforced surveillance of any one particular family

In addition to that there was such surveillance as occurred at the bleeding clinics, in the course of conversation, as well as the surveillance which occurred following the voluntary reporting of a symptom or a problem to the nurse

Thus our team, our operating team, had liaison with the families through the nurses We also had rather close working relationships with these families through their physicians or through their clinics, wherever and to whomever they turned for medical assistance in time of illness

A letter was written to each physician, each clinic, advising them of the family's participation in the project and inviting the physician or the clinic to use the public health nurse services as they saw fit, and to consult with us on any occasion upon which they deemed it necessary.

As a matter of fact, the office from which this study was administered was on the grounds of the village, but is was really a physical extension, as it were of the Health Department building itself, because its telephone was tied in with the Health Department's telephone on a common switchboard

Now, to secure freedom from bias we instituted what the trade knows as a "double-blind' study In other words, no one except one statistician knew the identity of those individuals who were receiving placebo and those who were receiving virus, and this identity was not revealed until the end of the study.

In the laboratory we attempted to achieve free dom from bias in interpreting results by a rather complex numbering and delivery system When a blood specimen reached the laboratory, it had on it the family's and the individual's code num ber, which was a combination of a family and an individual number but by the time it reached the laboratory area where the actual antibody titrations were done, this identifying code number had been replaced by a random and scrambled number, so that it was impossible for the laboratory personnel—that is the testing personnel—to identify any one specimen as to its individual origin its set origin, or its sequence within any one given set

In truth the laboratory crew which received the specimens, separated the serum and made the first dilution of one to four, was a different crew from the one that carried on from there This was a good experimental condition imposed on us by a shortage of space in the Health Department building

So much, then, for the design

I should like to pass on to another aspect of our problem which we considered vital and important, and this was an evaluation of the symptoms that occurred or might occur throughout the study period.

I must remind you of one thing again, and that is that all the data on illness was gathered without our knowing which of the complainers had received placebo or which had received vaccine.

Our preliminary work in the village led us to expect a great deal of illness in this village The villagers and the participants themselves volunteered the fact that there was always some kind of a cold or flu going around Pediatricians who had had the villagers for patients told us to expect the yearly episode of diarrhea, which they called the "G I crud"

In the next table I would like to point out our record of illnesses, as we got them through the control period

It should be noted that this is the only period in which we can validly compare the illnesses as they occurred in each group

We have left out certain things that quite obviously and patently have no relation to the experiment For example, we have left out the diagnosable, communicable diseases which, by this time, consisted mostly of mumps and chicken pox. We have also left out those items which are quite remote in their possible connection with virus, such as orthopedic defects, pregnancy, and other degrees of trauma

Then what is left here we have classified under four headings one a respiratory group, another a gastro-intestinal group, a third group in which the only presenting symptom was fever, without *any other findings or voluntary complaints*, and finally, the inevitable miscellaneous, or "other" group.

Under each one of these groups we have tabulated the illnesses week by week as they were reported to us, under the headings of those who received virus and those who received placebo (Tables 4a and 4b) *

As can be seen, there is some variation week by week with which these reports came in, under the respiratory heading It can also be seen that, even though there was some variation, there are other weeks when the incidences are almost or completely identical, or are very close

In other words, when the chips are down, when the incidence is at its peak, you cannot tell one group from the other.

The same is true in the gastro-intestinal diseases. There is some variation from time to time, as can be seen, but again in these last three (Table 4a), note how close the totals are from one group to another.

Then, as will be noticed, of the individuals who got the placebo, more complained of fever than those who got the virus And you will see also other symptoms that we recorded· one complaint of dizziness, one of headache, and one of soreness in the neck region, in the virus group; and in the placebo group there was one complaint of red blotches on the body (Table 4a).

One can take any one of these figures and compare them, 45 against 39, 50 against 37, or one can add them all up and come out with these same ratios *For the children, I can state that* none of these differences are statistically significant.

As for the adults (Table 4b), the results are very much the same; week by week variability, but with a tendency at certain times for an almost perfect identity of the number of cases

I remember now that the complaint of headache, *dizziness and stiff neck which occurred* in a placebo came from the one woman whom all the villagers agreed would be the one to come down with "polio" out of the whole study—one of the inevitable neurotics in the group

Again, if you compare the 33 against the 38, the 87 against the 69, and the totals for statistical significance, you will find that such significance does not exist

In other words, there is nothing that occurred in the way of illness not otherwise diagnosed that can be ascribed or attributed to the ingestion of the vaccine

Now there is another thing that I think one can notice on this table, and that is that there was a pattern to these illnesses You will notice that in both adults and children the respiratory diseases predominated in February, up to the very beginning of March. On the other hand, the diarrheal diseases predominated mostly among children and adults from March on to the end of the control period, at the last week shown

* See p 380

This pattern of illness was not peculiar to the village. Certainly no one is surprised to find respiratory disease during the month of February and early March in children, anyhow. But throughout the community, Minneapolis itself, the immediate neighborhood and, as a matter of fact, the entire state, even places 100 to 200 miles away from the site of the study were reporting outbreaks of diarrhea during the month of March. So that what occurred in the village was a reflection of what was occurring in other parts of the state, and was in no way connected with the administration of these viruses, although we were blamed in some quarters for starting an epidemic of diarrhea.

How we could do this from a distance of 200 miles without vaccine is an accomplishment that I will some day try to develop, if I can.

Now, a large item I would like to bring to your attention is a review of the results that were obtained on antibody titration when comparing the pre feeding antibody titers to the post feeding antibody titers.

In our paper these have been set out as a combination of a correlation square and a conversion table. We rather like the correlation square in Minnesota, and it does have a lot of advantages.

One can, for example, look at those correlation squares and, with a quick glance, immediately appraise the over-all general situation. Then if one has more time, a leisurely look will reward one with a great deal of detailed information.

Let me ask you just to glance at these correlation squares for children, the virus-fed children and the placebo-control children; I think you will be immediately struck with the gross difference in the appearance of the correlation squares (Tables 5a and 5b).*

The impact of that is really quite startling. But, in addition, we have prepared from the correlation squares a set of conversion tables which more or less summarize, in reduced space, the results of our antibody titrations.

Table 9, below, depicts those results for children and by Types 1, 2, and 3; and in each one there is the virus-fed group and the placebo-control group.

First we have shown the gross change, which means a change for the better, beginning with a titer of less than 4 on pre feedings.

For Type 1 it can be seen that we have such a change in almost 90 per cent of the virus-fed participants, and that in the placebo-control group just a little less than 6 per cent showed such a change.

Now, if we wish to be a little more exacting and consider only those changes which were accompanied by a two tube dilution, and in this case it would be a 16-fold rise, we go to the next column and we see that in that instance 70 per cent of the virus-fed converted from less than 4 to a titer of 16 or better. In the placebo-control group, such conversion was only to the extent of 3.5 per cent.

One can then, of course, go along, beginning with any titer up to 64, and see how the changes

* See pp 383 and 384

Table 9 Antibody Conversion Rates—Children

	Gross Change	Sixteen-Fold or Greater Rise in Titer			
	<4 to Any Titer	<4 to 16 or >	4 to 64 or >	16 to 256 or >	64 to 1024
Type 1					
Virus-fed	157/175 = 89.7%	123/175 = 70.3%	24/35 = 68.6%	6/16 = 37.5%	5/13 = 38.5%
Placebo-control	5/85 = 5.9%	3/85 = 3.5%	0/18 = 0.0%	0/8 = 0.0%	0/4 = 0.0%
Type 2					
Virus-fed	91/104 = 87.5%	79/104 = 76.0%	33/60 = 55.0%	26/39 = 66.7%	8/24 = 33.3%
Placebo-control	8/52 = 15.4%	2/52 = 3.8%	0/26 = 0.0%	1/22 = 4.5%	0/12 = 0.0%
Type 3					
Virus-fed	177/207 = 85.5%	145/207 = 70.0%	12/19 = 63.2%	4/10 = 40.0%	0/8 = 0.0%
Placebo-control	4/107 = 3.7%	1/107 = .9%	0/6 = 0.0%	0/4 = 0.0%	0/1 = 0.0%

So much, then, for the design

I should like to pass on to another aspect of our problem which we considered vital and important, and this was an evaluation of the symptoms that occurred or might occur throughout the study period

I must remind you of one thing again, and that is that all the data on illness was gathered without our knowing which of the complainers had received placebo or which had received vaccine

Our preliminary work in the village led us to expect a great deal of illness in this village The villagers and the participants themselves volunteered the fact that there was always some kind of a cold or flu going around Pediatricians who had had the villagers for patients told us to expect the yearly episode of diarrhea which they called the "G I crud"

In the next table I would like to point out our record of illnesses, as we got them through the control period

It should be noted that this is the only period in which we can validly compare the illnesses as they occurred in each group

We have left out certain things that quite obviously and patently have no relation to the experiment For example, we have left out the diagnosable, communicable diseases which, by this time, consisted mostly of mumps and chicken pox We have also left out those items which are quite remote in their possible connection with virus, such as orthopedic defects, pregnancy, and other degrees of trauma

Then what is left here we have classified under four headings one a respiratory group, another a gastro-intestinal group, a third group in which the only presenting symptom was fever, without any other findings or voluntary complaints, and finally, the inevitable miscellaneous, or "other" group

Under each one of these groups we have tabulated the illnesses week by week as they were reported to us, under the headings of those who received virus and those who received placebo (Tables 4a and 4b) *

As can be seen, there is some variation week by week with which these reports came in, under the respiratory heading It can also be seen that, even though there was some variation, there are other weeks when the incidences are almost or completely identical, or are very close

In other words, when the chips are down, when the incidence is at its peak, you cannot tell one group from the other.

The same is true in the gastro-intestinal diseases. There is some variation from time to time, as can be seen; but again in these last three (Table 4a), note how close the totals are from one group to another

Then, as will be noticed, of the individuals who got the placebo, more complained of fever than those who got the virus. And you will see also other symptoms that we recorded: one complaint of dizziness, one of headache, and one of soreness in the neck region, in the virus group, and in the placebo group there was one complaint of red blotches on the body (Table 4a).

One can take any one of these figures and compare them, 45 against 39, 50 against 37, or one can add them all up and come out with these same ratios For the children, I can state that none of these differences are statistically significant

As for the adults (Table 4b), the results are very much the same; week by week variability, but with a tendency at certain times for an almost perfect identity of the number of cases

I remember now that the complaint of headache, dizziness and stiff neck which occurred in a placebo came from the one woman whom all the villagers agreed would be the one to come down with "polio" out of the whole study—one of the inevitable neurotics in the group

Again, if you compare the 33 against the 38, the 87 against the 69, and the totals for statistical significance, you will find that such significance does not exist.

In other words, there is nothing that occurred in the way of illness not otherwise diagnosed that can be ascribed or attributed to the ingestion of the vaccine

Now there is another thing that I think one can notice on this table, and that is that there was a pattern to these illnesses. You will notice that in both adults and children the respiratory diseases predominated in February, up to the very beginning of March On the other hand, the diarrheal diseases predominated mostly among children and adults from March on to the end of the control period, at the last week shown

* See p 380

of construction for adults in Figure 3b.* Here again, using the same coding, is the picture in the placebo-control group, not quite so identical, but still closer in the placebo-control group than in the virus-fed group. Nothing, you will say, to get excited about, except I will ask you to notice that after feeding the virus, in all cases we have reduced the number of those to this extent here, to that extent there, and to this extent here *(indicating the number of those who start out with a titer of less than 4, in Figure 3b)*

As I mentioned to you before, this slight increase in area here, here, and here *(indicating Figure 3)*, apparently when measured by 16-fold increases, is enough to throw this into the statistically significant category

There is still another way to measure the total effect of feeding the virus on the population, and that is to consider the antibody picture as a whole, in which we look at the effect of the vaccine in completing the immune status of the participants against all three types of poliovirus

In order to do this, we have constructed, as shown in Table 11, a set of correlation squares arranged in such a fashion as to represent the total antibody picture of the participants

Beginning at the upper lefthand corner, we see first the worst possible picture—triple negative—*which then progressively improves to a double* negative, a single negative, and finally the perfect picture of antibodies to all three types, and the same is done for infants, children, and adults

The first thing that I want to call attention to generally, is the concentration of numerals and figures over to the righthand side of these correlation squares, indicating that after feeding the picture is more generally favorable in that there appears to be a concentration either of those who have antibodies to all three types or those who are only negative to a single type

Now, let us compare the worst and the best antibody status For example, *in 71 infants there* were 37 triple negatives at the beginning of the study There was only one at the end

In 183 children there were 52 triple negatives at the beginning of the study There were none at the end

In 284 adults there were 22 triple negatives at the beginning, and only 4 at the end

* See p 389

If we look at the opposite extreme, we see that at the beginning of the study we had among the infants only 4 who had antibodies to all three types, at the end of the study we had increased this to 44 Of the 183 children, at first we only had 32 who were perfect; we increased this to 150 And among the adults, we started with 132 and increased this to 170

This, then, reviewed in a number of ways, is the result of the antibody titrations, and I now want *to pass on to give a few brief notes on material* which has to do with our findings on examination of stools

As will be recalled, there were six stool specimens collected from each participant—the first *one at the very onset of the study, before virus* was fed I can report now that in none of those stools were any polioviruses found I can also report that an examination of the sewage that drained from this village in two places, taken within the week before the study, were also negative for polioviruses

Now, confining ourselves to the group that was fed virus during the first half of the study, the so-called Group B, we recovered virus, following feeding in from 9 to 13 per cent of the adults, the differences depending upon the type of virus In children we recovered virus in from about 40 to 60 per cent, *depending again on the type of* virus And in infants we recovered virus in from 60 to 87 per cent, again depending upon the virus type

This entire picture can be seen in Figure 4 in a graphical form, in which we have listed six sets of bar graphs representing the six stool specimens the percentages of virus recovery noted and the color and hatching code designating the different types of virus

Of course, after feeding the first type of virus the only type of virus we got was Type 2 and *it did have a little tendency to be found,* or continued to be found in the specimens after the next virus feeding

This tendency for continuance was slightly more evident for Type 1, which is cross-hatched *here This was after it was fed, and, as can be* seen, it continues to be found at least in the next three stool specimens

Finally, the virus which seemed to have the greatest propensity to show *itself in the stool*

have occurred, on the basis of 16-fold increments

There is an explanation for the occurrence of even the small percentages in the placebo group, which will become apparent later

For Type 2, we get results which are fairly comparable In the virus-fed group the figure is close to 90 per cent again—87 5 per cent gross conversions and 76 per cent 16-fold conversions, as compared to only 3 8 conversions in the control group

In Type 3 the situation is very much the same, 85 and better grossly and 70 per cent by 16-fold increment, and a comparison of these with the placebo-control group shows that there are wide differences between the virus-fed and the placebo-control groups, both for gross change and for 16 fold change, especially when beginning with a *titer of less than 4*

Table 10 shows the same type of calculations for the adults, and here one immediately sees that things are not quite so happy, magnitude-wise Namely, compare for the virus-fed group and the placebo-control group a gross change of 44.3 to 11 1 for Type 1, 54 to almost 18 for Type 2, 40 4 to 11 5 for Type 3

wide as in the case of the children—nevertheless, these modest magnitudes, when measured for statistical significance, curiously enough do turn out to be significant. In other words, even these differences, not so wide, are not accidental

Now, I *can present this same type of* data by a little different device, namely, by the construction of what we call antibody profiles, which are made by plotting on the ordinate the percentage of the pertinent population who have antibody titers of the magnitude that is plotted on the abscissal (Figure 3a) *

This is done for Type 1, Type 2, and Type 3 "Before feeding" is represented by the solid line, "after feeding" is shown by the broken line And for the placebo control group, one can practically superimpose the before and after antibody *profiles for all three types In other words*, the populations are identical from the antibody standpoint, before and after they receive the placebo

On the other hand, in those children who received virus, again one notices the same solid line, giving the same picture here, but what a difference has occurred with the broken line!

TABLE 10 ANTIBODY CONVERSION RATES—ADULTS

	Gross Change	Sixteen-Fold or Greater Rise in Titer			
	<4 to Any Titer	<4 to 16 or>	4 to 64 or>	16 to 256 or>	64 to 1024
TYPE 1					
Virus-fed	31/70 = 44 3%	11/70 = 15 7%	5/35 = 14 3%	5/50 = 10 0%	3/57 = 5 3%
Placebo-control	4/36 = 11 1%	0/36 = 0 0%	1/16 = 6 3%	2/26 = 7 7%	0/25 = 0 0%
TYPE 2					
Virus-fed	31/57 = 54 4%	12/57 = 21 1%	10/30 = 33 3%	14/55 = 25 5%	8/54 = 14 8%
Placebo-control	5/28 = 17 9%	1/28 = 3 6%	1/8 = 12 5%	1/27 = 3 7%	1/29 = 3 4%
TYPE 3					
Virus-fed	46/114 = 40 4%	25/114 = 21 9%	5/32 = 15 6%	4/35 = 11 4%	5/53 = 9 4%
Placebo-control	7/61 = 11 5%	0/61 = 0 0%	2/17 = 11 8%	0/16 = 0 0%	2/24 = 8 3%

However, turning to that part of the calculation where only the 16-fold increment are considered, one finds that these differences assume a somewhat different character Here one sees 15 7 against zero; 21 against something less than 4; and almost 22 against zero.

So that while these differences in absolute magnitude between the virus-fed and the placebo-control groups are not so wide—that is, not so

Immediately we note that the number of those with less than 4 titers has dropped, and, concurrently, those with titers above less than 4 has increased in what appears to be a weak attempt to stimulate the normal curve of distribution, with a marked difference between these two

Now we can see the same thing, the same type

* See p 388

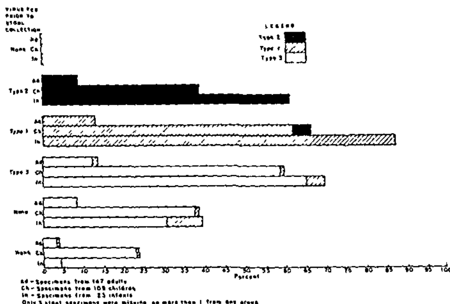


Fig 4 Percentages of poliovirus isolated from six stool specimens collected from group B individuals (vaccine group)

specimens on consecutive specimens was the Type 3 virus, whose finding is denoted by the unfilled bars

This, ladies and gentlemen, is the supply of virus, as nearly as we could denote it, which could be picked up by the control group during the first half of the study

Now, let me refer again to Table 2* merely to point out the opportunities that existed for picking up the virus by the control group from the the virus-fed group

Obviously, when you feed Type 2 here, you can pick up Type 2 in the second, third, and fourth specimen By the time you collect the fifth specimen, Type 2 is being fed here, of course *(indicating on Fig 4)*

This group can pick up Type 1 from this feeding in the third, fourth, and fifth stool specimens, at which time it itself will get Type 1, and Type 3 can be picked up in the last three specimens, by which time it itself will be fed Type 3

We had to cut off our work at this point here, we could not get a seventh specimen, desirable as it would seem, because at this time the university term ended and many of the villagers dispersed, went on vacation, or to some other activity

Table 12 gives a summary of the spread of viruses of the various types to the control group It can be seen that Type 2 spread to two adults, one child, and one infant, representing a total of three families and four individuals

TABLE 12 COMMUNITY SPREAD OF VIRUS TO GROUP A

		Virus Isolated		
Group A (Control)	Total No	Type 2 No	Type 1 No	Type 3 No
Adults	141	2	0	1
Children	95	1	11	9
Infants	30	1	1	3
Families	74	3	7	8

* See p 377

TABLE 11 THE EFFECT OF ORAL POLIOVIRUS VACCINE IN COMPLETING THE IMMUNE STATUS OF PARTICIPANTS AGAINST ALL THREE TYPES OF POLIOVIRUS

71 Infants

Pre-Vaccine	Post Vaccine: Triple Negative	Double Negative	Single Negative	Antibodies to all 3 types	Total No.	Total %
Triple Negative		2	13	22	37	52.1
Double Negative	1	3	5	11	20	28.2
Single Negative			2	8	10	14.1
Antibodies to all 3 types			1	3	4	5.6
Total Number	1	5	21	44	71	
%	1.4	7.0	29.6	62.0		100.0

183 Children

Pre-Vaccine	Post Vaccine: Triple Negative	Double Negative	Single Negative	Antibodies to all 3 types	Total No.	Total %
Triple Negative			19	33	52	28.4
Double Negative		1	11	54	66	36.1
Single Negative			2	31	33	18.0
Antibodies to all 3 types				32	32	17.5
Total Number		1	32	150	183	
%		0.5	17.5	82.0		100.0

284 Adults

Pre-Vaccine	Post Vaccine: Triple Negative	Double Negative	Single Negative	Antibodies to all 3 types	Total No.	Total %
Triple Negative	4	8	5	5	22	7.7
Double Negative		14	25	7	46	16.2
Single Negative		4	44	36	84	29.6
Antibodies to all 3 types			10	122	132	46.5
Total Number	4	26	84	170	284	
%	1.4	9.2	29.6	59.8		100.0

TABLE 14 COMMUNITY SPREAD TO GROUP A AS EVIDENCED BY ISOLATION AND/OR SEROLOGICAL RESPONSE

GROUP A (CONTROL)	TOTAL No	TYPE 2 No	TYPE 1 No	TYPE 3 No	TOTAL No	TOTAL %
Adults	141	5	3	5	11*	7 8
Children	95	1	11	9	21	22 1
Infants	30	1	1	3	5	16 7
Total Persons	266	7	15	17	37*	13 9
Families	74	6	9	11	24*	32 4

* 2 less than apparent total because 2 types of virus (involving 2 adults) reduce the person and family totals

in the antibody titrations and also give some notes on data which you are all anxious to get

One is, What is the persistence of these antibodies some months after the feeding? And the second item is concerned with what happens when one re feeds those participants who are apparent failures in the original study

DR BAUER: As Dr Kleinman has indicated, we were interested of course in the persistence of the antibodies after feeding So I will present some data showing the durability of the antibodies approximately eight months after the vaccine was fed to the vaccinees

The laboratory procedure which we used to obtain our data is described in detail in our manuscript which you have, and therefore I will not repeat it

However, I wish to re emphasize certain parts of our laboratory procedure which we feel are important in principle They are,

First That all blood specimens received in the laboratory were given a random code number

Second The laboratory personnel did not know which sera were from the same person

Third. They did not know whether they were testing serum from the first, second, third, or fourth blood specimen

Fourth They did not know whether the sera were from the control group or from the participants receiving the vaccine However, the serum specimens were so arranged so that all specimens from the same individual were tested on the same day and in the same test run

The neutralization test was used to determine the serum antibody titers for which the HeLa cell tissue culture and unattenuated Type 1 Mahoney, Type 2 MEF, and Type 3 Saukett strains of virus were employed.

The neutralization end point was expressed as the reciprocal of the highest dilution of serum, which completely neutralized the virus test dose

To determine the persistence of antibodies induced by the oral vaccine, we obtained blood specimens from volunteers who participated in the 1958 Como Village studies, which Dr Kleinman just described to you

Blood was obtained from 207 adults and children. These blood specimens were paired with blood serum collected eight months previously

Upon completion of the tests, we sorted out for comparative purposes the adults and children with no detectable antibodies at serum dilution 1 to 4 prior to the ingestion of attenuated virus vaccine

This group was chosen because it was the only group of whom we could be reasonably certain, as to the fact that whatever detectable antibody titers they might have were probably induced by the oral vaccine

Table 15 shows the changes in antibody titers in adults 1 to 4 and 9 to 12 months after the ingestion of the monovalent vaccine Only those adults who had no detectable antibodies in serum dilution 1 to 4 prior to feeding are shown on this table

You will recall that they had received each type of virus separately in capsules at intervals of 3 weeks, beginning with Type 2, followed by Type 1, and then by Type 3.

Type 1 spread to none of the adults but to 11 children and one infant, and these 12 individuals represent seven families So that we can thus conclude that there must have been some families in which more than one person showed the virus

For Type 3, there was one adult who picked it up, nine children, and three infants, or a total of 13 These 13 were distributed in eight families, and we can again conclude that there were some families in which more than one person showed the virus

Now, this is what we call community spread Remember, this is from one family to another, this is not intrafamilial spread, but interfamilial spread, primarily

There is another way in which we can deduce community spread, namely, if in our community control group we detected in the first two blood specimens a significant rise in antibody titer to one or more of the sero types.

Table 13 shows the evidence for community spread on the basis of antibody titer rise For Type 2 we have evidence of spread to 3 adults, with a pre-feeding titer of four, the post control titer in one individual who had not yet received any virus, was 256, and so it can be seen, for the rest of the Type 2 category, that there is good serological evidence that these people picked up an infection

The same is true for the three individuals in Type 1 and for the four in Type 3. As the asterisk shows here, we have case 47-1, who apparently got a double benefit serologically speaking; *and the same thing is true for case* 144-1, who also got a double benefit for Types 1 and 3 And, as is shown, Type 1 poliovirus was isolated from one child of case 47 1, and Type 3 poliovirus was isolated from the two children of case 144-1

In Table 14 we have combined the data, the evidence, for what we call interfamilial spread, counting together all of the persons on whom evidence exists for the spread either by virus isolation or serological response. As can be seen, there was evidence of the spread in 37 individuals in 24 families. So that something like 14 per cent of the control group showed evidence of having been infected by the fed group, and, of course, because the number of families is smaller, the percentage of families hit in the same way was 32 4

That is the extent to which I wish to speak to you, and I shall give the rest of the time over to my colleague, Dr. Bauer, who will tell you something about the laboratory procedures used

TABLE 13. SEROLOGICAL EVIDENCE (16 FOLD OR GREATER RISE IN TITER) OF COMMUNITY SPREAD WHICH WAS NOT CONFIRMED BY VIRUS ISOLATION

Poliovirus Type	Individual Identity			Antibody Titer		
	Family No — Person No	Sex	Age	Pre-	Post Control	Post Vaccine
2	43-1	Ma	25	4	256	64
	56-1	Ma	31	64	1024	256
	69-2	Fe	27	<4	16	4
1	47-1*	Ma	26	16	256	256
	110-1	Ma	33	4	64	16
	144-1†	Ma	25	16	256	64
3	5-2	Fe	28	4	64	4
	33-2	Fe	26	64	1024	256
	47-1*	Ma	26	64	1024	256
	144-1†	Ma	25	4	64	64

* Type 1 poliovirus isolated from 1 child of 47-1.
† Type 3 poliovirus isolated from 2 children of 144 1

A few words to orient you to the correlation squares. On the horizontal scale reading from left to right are the serum dilutions prepared with serum specimens collected 9 to 12 months after feeding of the virus. If a serum diluted 1 to 4 failed to demonstrate neutralizing antibodies for a particular type of virus, we concluded that the serum antibody titer was less than 4. Accordingly, the titers on the horizontal scale read from left to right less than 4, 4, 16 etc., through 1024 and on the vertical scale they read from top to bottom less than 4, etc., through 1024 for blood specimens collected 1 to 4 months after feeding. All figures which appear on the diagonal line represent persons who showed no change in antibody titer for the period measured; the figures which appear below the line represent a drop in titer and those appearing above the line represent a rise in titer. I do not intend to discuss the individual figures within the correlation square. I will confine my discussion to the gross changes which occurred to the group. Let us examine the correlation square for Type 1 antibody. We find 23 adults with an antibody titer of less than 4 prior to the ingestion of Type 1 virus. One to 4 months after ingesting the virus we find antibodies in serum dilutions ranging from 4 through 256 for 8 of the 23 adults. On examining their antibody titers 9 to 12 months later, we find that 4, or 50 per cent of the 8 retained a titer ranging from 4 through 16.

When we examine the antibody response to Type 2 virus, we find 20 adults with an antibody titer of less than 4; however, 1 to 4 months after ingesting the vaccine 10 of the 20 showed antibody titers ranging from 4 through 256. Nine to 12 months later we find 8, or 80 per cent, retaining a titer ranging from 4 through 16.

For Type 3 antibody titer we have 46 persons with a pre-feeding antibody titer of less than 4, and 1 to 4 months later 20 responded to the vaccine with antibody titers ranging from 4 through 64. Nine to 12 months later we find 11, or 55 per cent, maintained their titers and they ranged from 4 through 16.

You will note that I excluded from our group those individuals whose antibody titer was less than 1 to 4, 1 to 4 months after ingestion of the vaccine, and then showed an increase in titer 9 to 12 months later. I do not feel that we can accept these titers to be due to the vaccine we fed.

I shall now refer to Table 16.

Like the adults, the children were fed the three types of virus separately at intervals of 3 weeks. Only children who had no detectable antibodies at serum dilution of 1 to 4 prior to feeding of the vaccine are included in this study. In examining the antibody response of the 65 children who had a Type 1 pre-feeding antibody titer of less than 4, 61 responded to the stimulus of the oral vaccine with antibody titers ranging from 4 through 256. Nine to twelve months later we have 46, or 75 per cent, who retained their titers ranging from 4 through 256.

With regard to Type 2 we find 42 children with a pre-feeding antibody titer of less than 4, and 1 to 4 months later after feeding 35 children show an antibody response ranging in titer from 4 through 1024. Nine to 12 months later we find 25, or 71 per cent, with titers and these range from 4 through 256.

Examination of our correlation square for Type 3 antibodies shows us that there are 81 children who had a pre-feeding titer of less than 4, and 1 to 4 months later after ingesting Type 3 virus, 67 children responded yielding titers ranging from 4 through 256. When we look at their titers 9 to 12 months later, we find 42, or 63 per cent, with antibody titers of 4 through 1024.

Before drawing conclusions on the data presented, I believe it is necessary to inform you that we have some data which indicates that the persistence of antibodies in individuals with previous experience with killed vaccine and subsequently fed oral vaccine is essentially the same as for people who had no previous experience with killed vaccine and were subsequently fed live virus.

I believe we can state that orally administered live attenuated poliovirus stimulates the production of antibodies, and even though there is a general drop in titer from the originally induced titer, the antibodies persisted for 8 months in 50 to 80 per cent of the adults, and in 63 to 75 per cent of the children tested.

We have another bit of information which may be of interest to you. We were concerned with the individuals who were fed virus vaccine and for whom we could not demonstrate antibodies

TABLE 15. CHANGES IN ANTIBODY TITER WITH TIME AFTER FEEDING MONOVALENT VACCINE (TYPES FED SEPARATELY) INCLUDING ONLY ADULTS WHO HAD NO ANTIBODIES DETECTABLE AT SERUM DILUTION 1·4 PRIOR TO VACCINE FEEDING

Titer 9 - 12 Months After Feeding

Type 1

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	15						15		
4	3	2					5	60.0%	
16	1	1					2	100.0%	50.0%
64									
256			1				1	100.0%	100.0%
1024									
Totals	19	3	1				23		

Type 2

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	9	1					10		
4	1	3					4	25.0%	
16	1	1					2	100.0%	50.0%
64		1	2				3	100.0%	33.3%
256			1				1	100.0%	100.0%
1024									
Totals	11	6	3				20		

Type 3

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	24	1		1			26		
4	6	5	1				12	50.0%	
16	3	2	1				6	83.3%	50.0%
64		1	1				2	100.0%	50.0%
256									
1024									
Totals	33	9	3	1			46		

A few words to orient you to the correlation squares On the horizontal scale reading from left to right are the serum dilutions prepared with serum specimens collected 9 to 12 months after feeding of the virus. If a serum diluted 1 to 4 failed to demonstrate neutralizing antibodies for a particular type of virus, we concluded that the serum antibody titer was less than 4 Accordingly, the titers on the horizontal scale read from left to right less than 4, 4, 16, etc, through 1024 and on the vertical scale they read from top to bottom less than 4, etc., through 1024 for blood specimens collected 1 to 4 months after feeding All figures which appear on the diagonal line represent persons who showed no change in antibody titer for the period measured, the figures which appear below the line represent a drop in titer and those appearing above the line represent a rise in titer. I do not intend to discuss the individual figures within the correlation square I will confine my discussion to the gross changes which occurred to the group Let us examine the correlation square for Type 1 antibody We find 23 adults with an antibody titer of less than 4 prior to the ingestion of Type 1 virus One to 4 months after ingesting the virus we find antibodies in serum dilutions ranging from 4 through 256 for 8 of the 23 adults On examining their antibody titers 9 to 12 months later, we find that 4, or 50 per cent of the 8 retained a titer ranging from 4 through 16

When we examine the antibody response to Type 2 virus, we find 20 adults with an antibody titer of less than 4, however, 1 to 4 months after ingesting the vaccine 10 of the 20 showed antibody titers ranging from 4 through 256 Nine to 12 months later we find 8, or 80 per cent, retaining a titer ranging from 4 through 16

For Type 3 antibody titer we have 46 persons with a pre-feeding antibody titer of less than 4, and 1 to 4 months later 20 responded to the vaccine with antibody titers ranging from 4 through 64 Nine to 12 months later we find 11, or 55 per cent, maintained their titers and they ranged from 4 through 16

You will note that I excluded from our group those individuals whose antibody titer was less than 1 to 4, 1 to 4 months after ingestion of the vaccine, and then showed an increase in titer 9 to 12 months later I do not feel that we can accept these titers to be due to the vaccine we fed

I shall now refer to Table 16

Like the adults, the children were fed the three types of virus separately at intervals of 3 weeks Only children who had no detectable antibodies at serum dilution of 1 to 4 prior to feeding of the vaccine are included in this study In examining the antibody response of the 65 children who had a Type 1 pre feeding antibody titer of less than 4, 61 responded to the stimulus of the oral vaccine with antibody titers ranging from 4 through 256 Nine to twelve months later we have 46, or 75 per cent, who retained their titers ranging from 4 through 256

With regard to Type 2 we find 42 children with a pre-feeding antibody titer of less than 4, and 1 to 4 months later after feeding 35 children show an antibody response ranging in titer from 4 through 1024 Nine to 12 months later we find 25, or 71 per cent, with titers and these range from 4 through 256.

Examination of our correlation square for Type 3 antibodies shows us that there are 81 children who had a pre feeding titer of less than 4, and 1 to 4 months later after ingesting Type 3 virus, 67 children responded yielding titers ranging from 4 through 256. When we look at their titers 9 to 12 months later, we find 42, or 63 per cent, with antibody titers of 4 through 1024

Before drawing conclusions on the data presented, I believe it is necessary to inform you that we have some data which indicates that the persistence of antibodies in individuals with previous experience with killed vaccine and subsequently fed oral vaccine is essentially the same as for people who had no previous experience with killed vaccine and were subsequently fed live virus

I believe we can state that orally administered live attenuated poliovirus stimulates the production of antibodies, and even though there is a general drop in titer from the originally induced titer, the antibodies persisted for 8 months in 50 to 80 per cent of the adults, and in 63 to 75 per cent of the children tested

We have another bit of information which may be of interest to you We were concerned with the individuals who were fed virus vaccine and for whom we could not demonstrate antibodies

TABLE 15. CHANGES IN ANTIBODY TITER WITH TIME AFTER FEEDING MONOVALENT VACCINE (TYPES FED SEPARATELY) INCLUDING ONLY ADULTS WHO HAD NO ANTIBODIES DETECTABLE AT SERUM DILUTION 1:4 PRIOR TO VACCINE FEEDING

Titer 9 - 12 Months After Feeding

Type 1

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	15						15		
4	3	2					5	60.0%	
16	1	1					2	100.0%	50.0%
64									
256			1				1	100.0%	100.0%
1024									
Totals	19	3	1				23		

Type 2

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	9	1					10		
4	1	3					4	25.0%	
16	1	1					2	100.0%	50.0%
64		1	2				3	100.0%	33.3%
256			1				1	100.0%	100.0%
1024									
Totals	11	6	3				20		

Type 3

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer: Gross	Fall in Titer: 16x or >
<4	24	1		1			26		
4	6	5	1				12	50.0%	
16	3	2	1				6	83.3%	50.0%
64		1	1				2	100.0%	50.0%
256									
1024									
Totals	33	9	3	1			46		

in serum dilution 1 to 4 for one or more types of virus Accordingly, 30 adults and 14 children who had been fed the monovalent vaccine in 1958 were re-fed in 1959 with a trivalent vaccine None of the 14 children, and only 2 of the 30 adults, had no detectable antibodies at serum dilution 1 to 4 to all three types of virus The trivalent liquid vaccine was fed once in a 2 cc. dose, each dose containing 1,200,000 tissue culture doses per strain of virus fed They were bled 3 to 4 weeks after ingestion of the trivalent liquid vaccine

bettering in antibody titer to Type 1 antigen; 44 per cent showed an increase in titer to Type 2 antigen, and 77.3 per cent of the adults with an antibody titer of less than 4 for Type 3 showed an increase in antibody titer after ingesting the trivalent vaccine When measured by the stricter, and from the laboratory standpoint, a more significant criterion of 16-fold or greater rise in titer, we find 80 per cent fall in this category for Type 1, 10 per cent for Type 2, and 45 5 per cent for Type 3

With regard to the antibody response in chil-

TABLE 17. ANTIBODY RESPONSE TO TRIVALENT VACCINE IN 1959 SELECTED INDIVIDUALS, FED MONOVALENT VACCINE IN 1958 (TYPES FED SEPARATELY) WITHOUT ANTIBODY RESPONSE

Poliovirus Type	30 Adults <1 4 Pre-refeeding	Titer After Feeding Trivalent Vaccine						Total Change No	Positive Gross %	Response 16x or> %
		<4	4	16	64	256	1024			
1	15	3		6	6			12	80 0	80 0
2	10	6	3				1	4	40 0	10 0
3	22	5	7	6	3	1		17	77 3	45 5

Poliovirus Type	14 Children <1 4 Pre-refeeding	Titer After Feeding Trivalent Vaccine						Total Change No	Positive Gross %	Response 16x or> %
		<4	4	16	64	256	1024			
1	3				3			3	100 00	100 0
2	7	4	2		1			3	42 9	14 3
3	6	2	1	2	1			4	66 7	50 0

I might say, we have reason to believe that the two adults who were triple negative probably did not take the vaccine in the capsule form when our study was going on at the village

As you can see in Table 17, 15 of the 30 adults had no detectable antibodies in serum dilution of 1 to 4 for Type 1, 10 of the 30 had no detectable antibody titer in serum dilution of 1 to 4 for Type 2, and 22 had no detectable antibodies in serum dilution of 1 to 4 for Type 3

After feeding of the trivalent vaccine, we find 80 per cent of the adults who had an antibody titer of less than 4 for Type 1 elicit an over-all

dren who were fed the trivalent vaccine, we find a 100 per cent gross and 16-fold or greater increase to Type 1 antigen, 42 9 per cent elicited a gross, and 14 3 per cent a 16-fold or greater rise in antibody titer to Type 2 antigen; 66 7 per cent yielded a gross and 50 per cent a 16-fold or greater rise in antibody titer to Type 3 antigen

I believe we have placed a strict requirement on ourselves in respect to the antibody titers and response to the antigen In conclusion, we have great confidence in what we have achieved with the vaccine we fed to the participants in our study.

Thank you

TABLE 16 CHANGES IN ANTIBODY TITER WITH TIME AFTER FEEDING MONOVALENT VACCINE (TYPES FED SEPARATELY) INCLUDING ONLY CHILDREN WHO HAD NO ANTIBODIES DETECTABLE AT SERUM DILUTION 1.4 PRIOR TO VACCINE FEEDING

Titer 9 - 12 Months After Feeding

Type 1

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer Gross	Fall in Titer 16x or >
<4	4						4		
4	6	5	1				12	50.0%	
16	4	8	6	3			21	57.1%	19.0%
64	4	12	2	3	1		22	81.8%	72.7%
256	1	4	1				6	100.0%	100.0%
1024									
Totals	19	29	10	6	1		65		

Type 2

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer Gross	Fall in Titer 16x or >
<4	6			1			7		
4	3						3	100.0%	
16	4	5	1				10	90.0%	40.0%
64	3	7	4	2	1		17	82.4%	58.8%
256		1	2	1			4	100.0%	75.0%
1024				1			1	100.0%	100.0%
Totals	16	13	7	5	1		42		

Type 3

1 - 4 Months After Feeding	<4	4	16	64	256	1024	Totals	Fall in Titer Gross	Fall in Titer 16x or >
<4	11	1	2				14		
4	2	1		1	2		6	33.3%	
16	16	7	2	2			27	85.2%	59.3%
64	4	9	2	2	1		18	83.3%	72.2%
256	3	5	3	1			12	100.0%	91.7%
1024			2	2			4	100.0%	100.0%
Totals	36	23	11	8	3		81		

which apparently showed the least reaction of our strains from the standpoint of pathology, as indicated by Dr Melnick's and Dr. Murray's groups, was the type that spread the most

Frankly, I believe that perhaps in the final analysis our Type 2 strain is the most altered virus strain described so far

DR ANDERSON. I am glad Dr. Bodian commented on the low degree of spread, and I should like to emphasize what Dr. Kleinman said regarding the degree of congestion in this area This is really a highly congested area

These people are living in very cramped quarters and there is a great deal of visiting back and forth. The children are in and out of each other's homes constantly, the adults themselves are in and out of each other's homes, and if any one of the couples wants to go out for an evening, they have to exchange baby sitters The children are constantly mingling with one another

They are young children who, essentially, are below the age at which you can expect any level of sanitary consciousness, and yet in spite of that there was a very low amount of spread under these conditions

DR BARR The problem of setting up a control study in a group of this type would be very much simpler to conduct again in Minnesota, than it was the first time

There were two factors one was the concern, really serious concern, about hazards of the use of the vaccine, and the second was that one or two individuals did broadcast their view that the vaccine was very dangerous We had to lay the cards right on the table, with all these people, to make clear exactly what had happened up to that time in the opinion of the people who were working with it, as to the hazards in connection with the vaccine

I am sure that in a further study, instead of reaching 550 out of 1,000 inhabitants, we would have wound up with 700 or 800 out of 1,000 in the village

We have one other study that has just now started

DR KLEINMAN We have just completed the operation of part of a study in the village, comprising 220 people, in which we are trying to assess the effectiveness of the simultaneous feeding of all three types

This is also being controlled, in that a randomly selected unknown group is receiving monovalent vaccine, in three separate doses, and the trivalent group is receiving two doses of placebo and, as a final dose, the trivalent.

We have also made some attempts, rather modest, to demonstrate viremia and pharyngeal excretion in this group

A third study, which takes in a population in which the individuals are as isolated as we could possibly get them, except those who are in death cells awaiting execution, comprises 170 individuals in a prison in Minnesota Our main effort here, where we are using the trivalent vaccine again, will be further studies on attempts to demonstrate viremia and its relation to pharyngeal and fecal excretion This study is also controlled in that one entire cell house is getting placebo, and there are placebo controls scattered through each of four other cell houses

We have completed the taking of pharyngeal swabs and venous blood specimens on the third, fifth, and seventh day after feeding

The operational part of this study will be completed by the middle of July, and we are very anxious to get a large population where we can set up something similar to the study that we carried out first in the village

We think it can be done It may be difficult, but it can be done

DISCUSSION

CHAIRMAN GEAR: The floor is now open for discussion

DR BELL We cannot let this opportunity go without congratulating the authors on their carefully controlled study, we certainly do appreciate studies which are so meticulously planned and carried out

I have only one small comment and one question In comment, Dr Kleinman found no evidence that the vaccine caused or prevented any illness, but there were many illnesses and some of these must have been virus infections Therefore, the small numbers studied rather intensively suggest that there was no evidence of virus interference, otherwise there should have been fever illnesses in the vaccinated group

I am wondering you tested the individuals who had no antibody response one year later Did you also bleed and test some of those who did have a response, to see how well the antibody response was maintained for a year?

DR BAUER We have only tested what was reported We have not gone beyond that period of time

DR. KLEINMAN I mentioned that we had not isolated any polioviruses from the stool specimens collected before the actual operation of the study.

We did isolate, out of all this mass of stool specimens, 14 adeno-viruses

We have gone back now to see whether or not there might have been some viruses present in this community, which would not come out on HeLa cells, and up to now we have completed probably 150 on monkey kidney tissue cultures looking particularly for ECHO viruses and have found none.

We were particularly interested in finding out whether our failures might have been due to interference by a virus which we did not pick up on our routine HeLa cells; but in all of the failures that we tested we found no evidence of ECHO viruses, and, as a measure of control, we tested some 20 who did respond to the virus feedings, and in those we also found no ECHO viruses

DR. BODIAN: I think it is rather impressive that a control study can be put on with a live virus vaccine, and I was seeking for something that came out of it that could contribute information we had not had before.

Of course the study is small, but one of the things that impresses me is the evidence for a relatively low degree of spread to the control group in a population which seems to be sociable and perhaps congested, and it brings up a question that I raised some years ago, as to whether in the communities which need immunization most of all, conditions are predisposed least of all to permit spread

This might be a good thing in some respects with a live virus vaccine, because it is possible that spread imposes risks as well as a "free ride"

I wonder whether we should not revise our thinking in relation to the ability to carry out controlled studies I am sure that this is not always as easy as it was in Minnesota, but I do not think we should be pessimistic, in view of the fact that there is usually ample compensation for all the effort necessary for control studies, whereas large uncontrolled studies are very difficult to interpret

DR. COX I would like to point out at this time that we did not undertake any additional studies until we were assured by the results of the Minnesota trials that we might proceed with a reasonable degree of safety. For instance, the work in Andes, Colombia, was started only after we had seen the Minnesota data

Although I realize that the number of subjects in the Minnesota trial was small, I would like to emphasize that Type 2 virus, which has had by far the most laboratory manipulation of any of our strains, has shown the least amount of familial or community spread. Type 1, which has undergone almost as much laboratory manipulation, showed slightly more spread Type 3,

365 registered stillbirths, in 1958 there were 18,459 births in hospitals with only 622 registered stillbirths. It is remarkable that more than 98% of births occur in a hospital.

The medical staff on duty in Leopoldville consists of 94 qualified physicians, i e, 1 per 3,000 109 European nurses, 31 sanitarians, 25 African medical auxiliaries, and 115 African dressers The number of beds available in Leopoldville hospitals is 3 235, i e, 1 per 100

There is also a medical laboratory in Leopoldville with the most modern equipment· the Institut de Medecine Tropicale Princesse Astrid (The Princess Astrid Institute for Tropical Medicine). In 1958 this laboratory employed 6 physicians, 3 pharmaceutical chemists or biologists 13 sanitarians, and 89 African auxiliaries and workers

The virology section has been functioning for only two years Results of its work will be presented later

The city Health Service consists of 1 physician, 11 sanitarians, 35 Congolese auxiliaries and dressers, and 1,260 workers distributed throughout the various sectors

With such an organization, it is possible to make a fairly accurate survey of the epidemiological situation in Leopoldville

Epidemiology

The law prescribes that every physician must inform the public health officer of the number of cases of the contagious diseases (specified in legal texts) that he encounters each week.

Every year since 1951, in the case of polio, the physicians of Leopoldville were requested to give complete information on the identity of the patient, the size and nature of his family, and the type of paralysis. Hence we were able to gain accurate knowledge of the epidemiological pattern of the disease in Leopoldville.

For a general picture of public health in Leopoldville, Table 1 gives fairly clear evidence of the improvement of the conditions in our capital· it is enough to compare the increase in population with the concomitant decrease in general mortality and over all infant mortality It is interesting to note that Payne and Paul found an inversely proportional relation between the rate of general infant mortality, which is an indication of the state of hygiene and the occurrence of poliomyelitis

TABLE 1

YEAR	POPULATION	GENERAL MORTALITY RATE °/00	LIVE BIRTHS	INFANTILE MORTALITY RATE °/00
1950	190,912	135	6,473	197
1951	221,757	122	7,330	193
1952	244,152	130	7,942	187
1953	268,452	131	9,132	168
1954	282,760	100	10,167	160
1955	290,377	97	11,252	145
1956	331,488	79	15,436	87
1957	341,435	83	16,136	74
1958	320,583	87	18,459	82

The Public Health Institute, "Marcel Wanson," employs 3 physicians, 6 sanitarians, 6 European nurses, and 7 Congolese auxiliaries and dressers

The city Public Health Service is the branch "on the field" of the "Institut d'Hygiene Marcel Wanson," a laboratory carrying out studies in the field of public health generally and of epidemiology in particular

Although it is difficult to obtain accurate data prior to World War II on this illness in the tropics, we know that poliomyelitis has been present in Leopoldville for a long time The general epidemiological situation in Leopoldville is shown in Table 2, which gives the number of cases of the principal diseases in 1958

5. PRELIMINARY REPORT ON MASS VACCINATION WITH LIVE ATTENUATED POLIOMYELITIS VIRUS IN LEOPOLDVILLE, BELGIAN CONGO

A. LEBRUN, M.D., J. CERF, M.D., H. M. GELFAND, M.D., G. COURTOIS, M.D., AND H. KOPROWSKI, M.D.*

DR. LEBRUN *(presenting the paper)*. For several years the Public Health Institute has been faced with an imperative task—solving the problem of endemic poliomyelitis After the successful mass vaccinations with attenuated live virus in 1957 by G Courtois and others of nearly 250,000 persons in Ruanda-Urundi and other communities in the Belgian Congo, the Public Health Institute decided to vaccinate the susceptible population of Leopoldville It was hoped that the vaccination campaign, in addition to achieving its objective of immunizing the population, would also yield further knowledge concerning certain aspects of the live vaccine particularly in regard to its effectiveness and safety These studies are not yet completed, nevertheless, they have already provided some interesting information

This presentation is intended to describe the background, the organization, and the preliminary results of these studies.

The Environment

The climate of Leopoldville is tropical. there are a cool, dry season from June to September, when the average temperature ranges from 64°F to 82°F., and the relative humidity from 53% to 94%, and a hot and humid season from October to May, when the average temperature ranges from 72°F to 87°F., and the relative humidity from 58% to 96% The nearest important townships are Matadi, 400 kms. away by rail or road, Coquilhatville, 600 kms. up the river, and Brazzaville (French Equatorial Africa, now the Congo Republic) on the other side of the river only 4 kms. away

It should be remembered that 1 million travellers come through Leopoldville every year

The rapid growth of the town, from 26,000 inhabitants in 1935 to 320,000 in 1958, raises the enormously difficult problems of urbanization. It also explains the lack of a complete sewer network, as well as the fact that alongside a so-called "New City," where housing is of a fairly progressive type, there still exists an "Old City" where Africans live in usually overcrowded houses built with the customary materials of primitive dwellings: mud walls and thatched roofs

However, housing conditions are fast improving Since 1950, for instance, more than 18 000 houses have been built of permanent materials in Leopoldville itself

Medical Control and Services

Every person is required to check once a year with the Medical Census Service in order to obtain a certificate stating that he does not have one of the great infectious diseases that formerly decimated the people of Africa sleeping sickness, tuberculosis, leprosy, and venereal disease This medical certificate is stamped in the identity book that each person carries The certificate must be renewed not more than a fortnight before one leaves on a journey lasting longer than three months During this yearly examination each person is vaccinated against smallpox In 1957, we also inoculated the entire population of Leopoldville with BCG, and in 1958 we began poliomyelitis vaccination in the approximately 75,000 children that were considered susceptible, i e, below 5 years old

Hospital services, too, have developed considerably The following statistics show this development and also indicate that the population is quite willing to accept modern medicine In 1945, 2,508 births occurred in hospitals, with

* Dr. Lebrun (Institute of Public Health "Marcel Wanson," Leopoldville), Dr Cerf (City Public Health Service, Leopoldville); Dr. Gelfand (Tulane University Medical School, New Orleans); Dr Courtois (Laboratoire Medicale, Leopoldville); and Dr Koprowski (The Wistar Institute, Philadelphia)

Tables 6, 7, 8, and 9 and graph 1 show the status of poliomyelitis during the past 9 years in Leopoldville.

The disease (Table 5) seems to appear clinically principally during the hot season. However, it develops both at the beginning and the end of that season the peaks of the epidemic were in April 1955 as well as in December 1958. The climatic factor, therefore, does not appear *a priori* to be predominant, at least in Leopoldville

Transmission by flies does not seem to be an important factor either Since the beginning of 1958, the fly infestation was at an all time low, but the epidemic at the end of that year was one of the two greatest since 1951.

The morbidity rate among Africans (Table 6), if we except 1954 when it was abnormally low, varies between 19 03 and 26 62 per 100,000; mortality remains low (under 5% of all cases) and sequelae are generally minor. For Europeans, on the contrary, even if morbidity is extremely variable, the mortality rate is high (20% of all cases), and the sequelae are regularly serious

TABLE 5 CASES REPORTED BY MONTHS AMONG AFRICANS OF LEOPOLDVILLE

YEAR	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
1951	19	10	0	1	3	5	10	2	2	0	1	1	54
1952	2	1	2	4	4	8	17	6	6	6	7	2	65
1953	10	3	1	2	1	1	4	4	6	9	15	5	61
1954	2	2	2	0	1	1	1	2	1	2	0	1	15
1955	7	6	12	23	7	6	3	3	3	3	1	—	74
1956	2	7	2	7	2	4	4	4	6	10	17	13	78
1957	10	5	2	5	3	1	0	5	8	3	8	15	65
1958	12	5	6	1	2	0	1	0	1	4	10	35	77
1959	25	15	7	5									

TABLE 6 LEOPOLDVILLE

YEAR		POPULATION	CASES	MORBIDITY 0/0000	DEATHS	MORTALITY 0/0
1951	E	10,965	1	9 11	0	0
	I	221,757	54	24 35	2	3 7
1952	E	13,275	5	37 66	1	20
	I	244,152	65	26 62	0	0
1953	E	15,649	1	6 39	0	0
	I	268,452	61	22 72	1	1 63
1954	E	15,225	4	26 27	1	25
	I	282,766	15	5 30	0	0
1955	E	15,883	19	119 62	4	21 05
	I	290,337	74	25 48	3	4 05
1956	E	19,117	4	20 —	0	0
	I	331,488	78	23 53	0	0
1957	E	19,101	3	15 —	0	0
	I	341,435	65	19 03	0	0
1958	E	19,668	1	5 —	0	0
	I	320,583	77	24 —	4	5 2

TABLE 2 EPIDEMIC DISEASES LEOPOLDVILLE

	1957	1958
Rickettsioses	1	2
Variola major	—	2
Pertussis	1678	4319
Diphtheria	51	29
Dysentery amebic	1125	545
Dysentery bacillary	291	1427
Paratyphoid Fever	28	121
Typhoid Fever	60	168
Flu	18147	4869
Meningitis	14	26
Mumps	1400	584
Rabies		1
Measles	1965	3682
Varicella	411	1587
Leprosy	76	260
Sleeping sickness		18
Yaws	51	12
Tuberculosis	342	795
Syphilis (all types)	485	842
Blennorhagia	2622	3687
Soft chancre	49	70
Granuloma inguinale	6	2
Lymphogranuloma venereum	1	13
Hepatitis serum	2	39
Hepatitis infectious	114	497
Amebic abcess of liver		3

Official documents from 1919 mention a not inconsiderable epidemic of polio that started in the lower Congo and was particularly active in the Leopoldville region It should be noted that this epidemic affected a large number of young adults and that there was a high mortality rate, which has not been approached since Rodhain reported the age distribution for 59 cases during the 1919 outbreak (see Table 3)

TABLE 3. AGE DISTRIBUTION FOR 59 CASES DURING 1919 OUTBREAK

AGE (YEARS)	NO CASES	PERCENT
½—2	15	25 4
3—7	27	45 7
8—12	3	5 0
15—20	2	
21	12	23 7

This distribution led Rodhain to draw the following conclusion:

"The large proportion of adult patients seems definitely to show that the epidemic had flourished on virgin ground or at least had developed in an environment that had not for a long time come into contact with the Heine-Medin virus."

Let us not forget that the virus was isolated by Rodhain in the monkey *Cercopithecus schmidti*, a remarkable feat for the time.

Only one case in (1924) was registered for the period April 1920 to 1934 I believe that this does not mean that the disease did not exist at that time The fairly small number of physicians were more concerned with fighting the great epidemics of smallpox and sleeping sickness than with reporting what seemed to be a minor disease for Africans.

As physicians became more numerous, the number of reported cases grew: this may reflect an actual increase in incidence of poliomyelitis The increase in poliomyelitis cases was probably due to the ever multiplying means of transport that helped to spread the virus throughout the country by means of more frequent contact.

Table 4 gives the number of cases registered between 1935 and 1958 for the entire Congo

TABLE 4 CASES OF POLIO AMONG AFRICANS LIVING IN BELGIAN CONGO

YEARS	NO OF CASES	DEATHS
1935	6	4
1936	52	6
1937	14	—
1938	11	1
1939	4	2
1940	3	—
1941	28	4
1942	12	2
1943	12	2
1944	20	1
1945	8	—
1946	32	3
1947	52	—
1948	86	2
1949	77	2
1950	326	18
1951	447	27
1952	592	22
1953	707	48
1954	710	39
1955	1447	77
1956	571	29
1957	564	31
1958	869	37

Tables 6, 7, 8, and 9 and graph 1 show the status of poliomyelitis during the past 9 years in Leopoldville

The disease (Table 5) seems to appear clinically principally during the hot season However, it develops both at the beginning and the end of that season: the peaks of the epidemic were in April 1955 as well as in December 1958 The climatic factor, therefore, does not appear *a priori* to be predominant, at least in Leopoldville.

Transmission by flies does not seem to be an important factor either Since the beginning of 1958, the fly infestation was at an all time low, but the epidemic at the end of that year was one of the two greatest since 1951.

The morbidity rate among Africans (Table 6), if we except 1954 when it was abnormally low, varies between 19 03 and 26 62 per 100,000, mortality remains low (under 5% of all cases) and sequelae are generally minor. For Europeans, on the contrary, even if morbidity is extremely variable, the mortality rate is high (20% of all cases), and the sequelae are regularly serious

TABLE 5 CASES REPORTED BY MONTHS AMONG AFRICANS OF LEOPOLDVILLE

YEAR	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL
1951	19	10	0	1	3	5	10	2	2	0	1	1	54
1952	2	1	2	4	4	8	17	6	6	6	7	2	65
1953	10	3	1	2	1	1	4	4	6	9	15	5	61
1954	2	2	2	0	1	1	1	2	1	2	0	1	15
1955	7	6	12	23	7	6	3	3	3	3	1	—	74
1956	2	7	2	7	2	4	4	4	6	10	17	13	78
1957	10	5	2	5	3	1	0	5	8	3	8	15	65
1958	12	5	6	1	2	0	1	0	1	4	10	35	77
1959	25	15	7	5									

TABLE 6 LEOPOLDVILLE

YEAR		POPULATION	CASES	MORBIDITY o/oooo	DEATHS	MORTALITY o/o
1951	E	10,965	1	9 11	0	0
	I	221,757	54	24 35	2	3 7
1952	E	13,275	5	37 66	1	20
	I	244,152	65	26 62	0	0
1953	E	15,649	1	6 39	0	0
	I	268,452	61	22 72	1	1 63
1954	E	15,225	4	26 27	1	25
	I	282,766	15	5 30	0	0
1955	E	15,883	19	119 62	4	21 05
	I	290,337	74	25 48	3	4 05
1956	E	19,117	4	20 —	0	0
	I	331,488	78	23 53	0	0
1957	E	19,101	3	15 —	0	0
	I	341,435	65	19 03	0	0
1958	E	19,668	1	5 —	0	0
	I	320 583	77	24 —	4	5 2

TABLE 7 POLIO IN LEOPOLDVILLE—DISTRIBUTION OF CASES BY AGE—AFRICAN POPULATION

YEAR	A					G			E			NO AGE	
	—1	1	2	3	4	5	6	7	8	11	25	LISTED	TOTAL
1951	21	16	12	1	2	1		1					54
1952	17	34	8	1		3						2	65
1953	20	30	7	2		1				1			61
1954	5	8	1	1									15
1955	27	33	8	3	2						1		74
1956	23	40	8	4	1		1			1			78
1957	20	34	7				1			2	1		65
1958	27	31	11	2		1			1	1		3	77
Total	160	226	62	14	5	6	2	1	1	5	2	5	489

The age distribution of patients (Tables 7 and 8) also differs in the two population groups Among Africans, 96 1 per cent of the patients are in the under-5-year age group, and 92 4 per cent are in the under-3-year group, whereas among Europeans the incidence is 39.4 per cent in the under-5-year group and 31 5 per cent in the under-3-year group The scattering of cases in those more than five years of age is also completely different in the two groups From epidemiological considerations alone, we already may conclude that it is necessary to vaccinate only those under 5 years old in the African population, and one might even reasonably plan the vaccination of the under-3-year group only

Information on the types of poliomyelitis virus in Leopoldville is given in Tables 9 and 10 The Virology Section of the Medical Laboratory has been working for only two years, and our information in this field barely goes beyond that of 1957

TABLE 8 POLIO IN LEOPOLDVILLE—DISTRIBUTION OF CASES BY AGE—EUROPEAN POPULATION

YEAR	AGE																				
	1	1	2	3	4	5	7	8	9	11	23	25	26	27	28	29	31	35	47	56	TOTAL
1951													1								1
1952			1										1			1	2				5
1953												1									1
1954		1	1	1								1									4
1955		3	2		2	1		2	1	1	1		1	1	1			1	1	1	19
1956	1		2															1			4
1957							1					1					1				3
1958	1																				1
Total	2	4	6	1	2	1	1	2	1	1	1	3	3	1	1	1	3	2	1	1	38

TABLE 9 VIRUS CIRCULATING IN LEOPOLDVILLE—ISOLATION FROM STOOLS OF HEALTHY AFRICAN CHILDREN (BELOW 2 YEARS AGE) LEOPOLDVILLE 1957-1958
INSTITUTE MEDECINE TROPICALE "PRINCESSE ASTRID"—DR VANDEPUTTE

MONTH—YEAR	NO. OF STOOLS[1]	% +	POLIO			COXSACKIE					B NOT YET	ADENO	NOT
			1	2	3	A	B2	B3	B4	B5	IDENTIFIED		IDENTIFIED
October 1957	109	17		1	9	8	1					1	2
November "	108	37	2	3	14	6					5	8	2
December "	106	27	3	2	10	6	1	1			3		4
January 1958	101	45	7	1	7	1			1		2	11	16
February "	101	30	1	1	3	13		1				4	7
March "	92	20	3			2		4		1	4	3	2
April "	101		1		1	2		1			5	6	5
May "					3	3		6		2	2		20

[1] HeLa cells and baby mice.

TABLE 10 VIROLOGY (I M T.P A)
SERVICE DOCTEUR VANDEPUTTE

Annual Report 1957		
No of stools examined 528		
101 + : 18 Coxsackies		
10 polio	9 Type 1	
	1 Type 2	
73	Adeno	
	Coxs A	
Annual Report 1958		
No of stools examined 1182		
polio	13 Type 1	
	7 Type 2	
	39 Type 3	
Coxsackie	12 A	
	54 B	
Adeno 50		
Virus not identified 116		

Table 9 is a record of viruses that were isolated (HeLa cells and *baby* mice) during epidemiological studies in 1957 and 1958 from the stools of African children under the age of two years who did not show any clinical sign of virus infection It becomes apparent that at that time poliovirus Type 3 was in circulation in epidemic form We may note that during that time in Leopoldville, of the two poliovirus isolations from paralytic cases both were Type 1.

In June and July 1958 there was an epidemic of Coxsackie B_5 infections characterized by high fever, headaches, myalgia (mainly abdominal), and sometimes by paralysis or weakness of the extremities Eight strains were successfully isolated 5 from stools and 3 from cerebrospinal fluid

Plans for Mass Vaccination

In view of the epidemiological situation that has been described, the Hygiene Service has been planning vaccination of the susceptible population of Leopoldville since 1956 (Annex)

We preferred to use a live attenuated virus for the following reasons

(1) This method avoids calling Africans in for repeated injections and hence guarantees that there will be no people left at large who are insufficiently immunized

(2) Oral administration facilitates mass vaccination and precludes the transmission of other viruses through failure to change the needles on the syringe.

(3) This method simulates the natural processes of infection and immunization without paralysis.

(4) Just as with vaccination against smallpox and yellow fever, we hoped to produce immunization that would be both effective and lasting and that would not require booster doses in the future.

These advantages greatly outweigh two drawbacks. (1) that the vaccine must be kept on ice, which, incidentally, is easily done, and (2) that small children may spit out part of the dose—requiring constant attention throughout the mass vaccination and special training of those who feed the children

The CHAT strain Type 1 of Koprowski was obtainable in sufficient quantity in 1958 for the vaccination of the 75,000 and odd susceptible children in Leopoldville, i e., those under 5 years

A meeting of all the physicians in town was convened to inform them of the experiment They were required to report immediately every suspected case of poliomyelitis and to give complete information as follows complete identification of the patient, date of vaccination, if any, and the clinical symptoms Whenever possible, blood samples were to be taken, as well as stools and cerebrospinal fluid for examination at The Wistar Institute.

Vaccination was started 18 August 1958, and continues at present, the results reported here are as of the end of April, 1959

The vaccination team includes one physician, 2 nurses, 2 dressers, and two clerks They go throughout the city working in schools or dispensaries At the same time vaccination is being performed in the Medical Center by others

The population is called street by street by the administrative authorities the day before vaccination is planned The stock vaccine is divided in the laboratory of the Institute of Public Health into small vials, which are kept frozen The quantity of concentrated vaccine in each vial is such that the final titer of 300 ml of saline solution is 10^6 tissue culture doses$_{50}$ per ml One ml. of this solution is given orally by means of a semiautomatic syringe For children under 30 days of age, we use a solution ten times more

TABLE 7 POLIO IN LEOPOLDVILLE—DISTRIBUTION OF CASES BY AGE—AFRICAN POPULATION

YEAR	AGE —1	1	2	3	4	5	6	7	8	11	25	NO AGE LISTED	TOTAL
1951	21	16	12	1	2	1		1					54
1952	17	34	8	1		3						2	65
1953	20	30	7	2		1				1			61
1954	5	8	1	1									15
1955	27	33	8	3	2						1		74
1956	23	40	8	4	1		1			1			78
1957	20	34	7				1			2	1		65
1958	27	31	11	2		1			1	1		3	77
Total	160	226	62	14	5	6	2	1	1	5	2	5	489

The age distribution of patients (Tables 7 and 8) also differs in the two population groups Among Africans, 96 1 per cent of the patients are in the under-5-year age group, and 92 4 per cent are in the under-3-year group, whereas among Europeans the incidence is 39 4 per cent in the under-5-year group and 31 5 per cent in the under-3-year group The scattering of cases in those more than five years of age is also completely different in the two groups From epidemiological considerations alone, we already may conclude that it is necessary to vaccinate only those under 5 years old in the African population, and one might even reasonably plan the vaccination of the under-3-year group only.

Information on the types of poliomyelitis virus in Leopoldville is given in Tables 9 and 10 The Virology Section of the Medical Laboratory has been working for only two years, and our information in this field barely goes beyond that of 1957.

TABLE 8. POLIO IN LEOPOLDVILLE—DISTRIBUTION OF CASES BY AGE—EUROPEAN POPULATION

YEAR	AGE 1	1	2	3	4	5	7	8	9	11	23	25	26	27	28	29	31	35	47	56	TOTAL
1951													1								1
1952			1										1			1	2				5
1953												1									1
1954		1	1	1								1									4
1955		3	2		2	1		2	1	1	1		1	1	1			1	1	1	19
1956	1		2															1			4
1957							1					1					1				3
1958	1																				1
Total	2	4	6	1	2	1	1	2	1	1	1	3	3	1	1	1	3	2	1	1	38

TABLE 9 VIRUS CIRCULATING IN LEOPOLDVILLE—ISOLATION FROM STOOLS OF HEALTHY AFRICAN CHILDREN (BELOW 2 YEARS AGE) LEOPOLDVILLE 1957-1958
INSTITUTE MEDECINE TROPICALE "PRINCESSE ASTRID"—DR VANDEPUTTE

MONTH—YEAR	NO OF STOOLS	% +	POLIO 1	POLIO 2	POLIO 3	COXSACKIE A	COXSACKIE B2	COXSACKIE B3	COXSACKIE B4	COXSACKIE B5	B NOT YET IDENTIFIED	ADENO	NOT IDENTIFIED
October 1957	109	17		1	9	8	1					1	2
November "	108	37	2	3	14	6					5	8	2
December "	106	27	3	2	10	6	1	1			3		4
January 1958	101	45	7	1	7	1			1		2	11	16
February "	101	30	1	1	3	13		1				4	7
March "	92	20	3			2		4		1	4	3	2
April "	101		1		1	2		1			5	6	5
May "					3	3		6		2	2		20

HeLa cells and baby mice

7 Sera will be accumulated in flame-sealed ampoules kept in deep freeze, until ½ are obtained, then sent to Wistar in refrigerator box with "dry ice" List of Type-1 negatives and triple negatives to be returned to Leopoldville as quickly as possible
8 After 1-2 months, negative children only will be rebled
9 Complete bleeding record to be prepared in triplicate 1 to remain in Leopoldville, 1 to be air-mailed to Wistar, 1 to accompany sera shipped to Wistar.
10 *Note*. Reduce possibility of mislabelling by preparing numbers in advance, transfer to skin of mother or child after record prepared, transfer to bleeding vial after bleeding, transfer to serum ampoule after separating.

Health Inquiry

1. To be done by nurses at 3 times: at vaccination (pre-vac.), 1 week post-vaccination (V + 1), 2 weeks post vaccination (V + 2)
2. Nurses to work together at clinic during first week to agree on inquiry technique, then to rotate on schedule of pre-vac., V + 1. and V + 2 so that differences are equalized
3. Inquiry cards to be consecutively numbered, and to indicate sibling vaccinees by A, B, C, etc
4. Record number of siblings older than 4 years on card
5. Card should be prepared on all children less than 4 years, even if vaccination is not given for illness or other reasons (Note that sick children will not be brought to health census and will not be found, but will report to census elsewhere when well.)
6. Inquiry should include all children in family, whether or not vaccinated Inquiry is for purpose of recording symptoms significant enough to come to attention of mother No leading questions should be asked except as they refer to central nervous system.
7. Recording must be simple, for coding in English The following should be recorded.

Etat général (fièvre)	*Système nerveux*
Peau	Céphalis
Tête et cou	Raideur de muque
Thorax système respiratoire	Algies dorso lombaire
Abdomen systeme digestif (vomissement)	Douleurs musculaires
	Faiblesse musculaire
Syst Uro-genital	Paralysie musculaire
Squelette et système musculaire	Troubles émotifs

Indicate severity by:
1. Mild (barely noticeable)
2. Moderate (definitely sick child)
3. Severe (indicated for hospitalization)

8 On post vaccination visits, newly appearing C.N.S symptoms to be reported to Service d'Hygiene MD for examination and laboratory specimens as indicated.

Vaccination

1 Stock virus has been put up in 1 cc amounts in vials.
2. For general use, 1 vial diluted to 300 cc in saline in pre-chilled bottles. To be kept thereafter on ice for use during the same morning, only discard residue. Vaccine administered by automatic pipette, 1 cc per child
3 For infants less than 30 days old, 1 vial diluted to 20 cc. in medicine dropper bottle. 1 cc per child, may be kept for 2 days use
4 Do not vaccinate obviously sick children have "pick up" team, or instruct to return later *(unless serological study indicates conversions occur in "skips")*
5 Observe vaccinated children closely to see that vaccine is swallowed Repeat "feeding" as often as necessary.

Hospitalized "Polio" Cases

1 All cases to be reported to Service d'Hygiene, and diagnosis to be acceptable by public health medical doctor
2. Data should include all personal identification, age, sex locality, when vaccinated if so
3 Laboratory specimens
   Sera—Acute as early as possible, convalescent at 2 - 4 weeks
   Feces—At least 2 specimens on 2 different days
   Spinal fluid—If possible (sterile)
4 Keep frozen until shipped in lots to Wistar—with sero-survey specimens at first, then separately in plastic bottles with "dry ice" in sawdust-packed box

concentrated A certain proportion of the children were to be followed after vaccination, for the possible occurrence of minor illnesses For this purpose they were examined, and a card was filled out with the results of the examination and, of course, their complete identification If a blood sample is to be taken, adhesive tape with a code number is affixed to the arm and the identification noted in a book The blood is collected in a vacuum tube and the tape transferred to the tube, which is sent to the laboratory for determination of antibodies against poliovirus.

We planned to obtain serological data in one thousand children, we obtained prevaccination blood specimens from 1,321 children under 5 years of age to allow for loss Of these 75% had no antibodies against Type 1 poliovirus

Unavoidable difficulties in January 1959 supervened so that we were unable to perform as many rebleedings as had been planned For the same reason the activity of the vaccination team was temporarily slowed down, and all the work was carried out in the Medical Census Center Rebleeding was done in 490 children, Dr Plotkin will speak about that aspect of our work

One of the most severe epidemics of the last ten years started in October 1958, Dr Plotkin will analyze that outbreak and the follow-up of the 7,600 children vaccinated

I apologize for anticipating the presentation of my distinguished colleague, but I believe that it is my duty as one who is responsible for the public health of Leopoldville to state my conclusions

(1) That from the epidemiological point of view, the vaccinations in Leopoldville were of indisputable benefit, and

(2) That the attenuated virus has never shown any noticeable harmful effect in Leopoldville, we do not hesitate, therefore, to consider it well worthwhile, and we are planning to extend its use, little by little, to the whole of the Congo as possibilities permit One million children will soon be vaccinated in Ruanda Urundi and 200,000 in the lower Congo region

ANNEX

Suggested Procedure for Conduct of Live Virus Polio Vaccine (CHAT Strain, Pool 13) Field Trial in Leopoldville

General

Vaccine to be administered by districts, at annual health census, with population called to a central site such as a school building.

Polio vaccine to be added to established routine Estimated attendance is 100 450 children under 4 years, per day, 6 days per week Each district should be completed within 1-2 weeks Total child population of vaccination age estimated 45 - 50,000

Blood Collection

1. Venipuncture preferred, femoral or anticubital vein as indicated by age Le Blanc heel puncture unsatisfactory except in very young
2. Object is to obtain 1,000 serum specimens for (a) estimation of Type 1-negative and triple-negative children vaccinated and (b) percentage of sero-conversion following vaccination
3. At prevaccination bleeding, we will collect 1,200 specimens on assumption of 10 15% loss after vaccination Ideal distribution of 1,000, and goal distribution of 1,200:

6 months - 1 year	340 - 400
1 - 2 "	358 - 400
2 - 3 "	168 - 200
3 - 4 "	109 - 150
4 - 5 "	25 - 50
	1,000 - 1,200

4. Above should be divided into approximately equal parts. children from African housing areas and from "western" housing
5. In addition to above, try to obtain 1 - 200 sera from ages 6 months - 2 years in children not vaccinated, to determine if sero conversion is as great among "skips"
6. Since first area (African housing) will take 2 weeks, bleed only during first week, since vaccine viruses may spread. Then move to opposite end of city for the second area (Western housing) for 1 week of bleeding If all not yet obtained move to a third separated area, etc.

6. EPIDEMIOLOGICAL STUDIES OF THE SAFETY AND EFFICACY OF VACCINATION WITH THE CHAT STRAIN OF ATTENUATED POLIOVIRUS IN LEOPOLDVILLE, BELGIAN CONGO

STANLEY A. PLOTKIN, M.D. AND HILARY KOPROWSKI, M.D.

The Wistar Institute
Philadelphia, Pennsylvania

DR PLOTKIN *(presenting the paper)* On 18 August 1958, a campaign of vaccination with the CHAT Type 1 attenuated poliovirus of Koprowski[1] was begun in Leopoldville, Belgian Congo The history of poliomyelitis in Leopoldville and the development and design of the campaign have just been presented to you by Dr Lebrun, *et al*[2] The present paper is designed to give detailed epidemiological data concerning the progress of the trial up to April 30, 1959, by which date over 45,000 children had already been vaccinated We shall concern ourselves principally with vaccine safety as determined by post-vaccination inquiries, and with the analysis of an epidemic of paralytic poliomyelitis which occurred during the trial

Susceptibility of the Population

Table 8 of the previous paper[2] shows that most paralytic poliomyelitis among Africans in Leopoldville occurs within the first two years of life, with 97% of cases occurring in children under 5 years From these data one could predict that antibodies to poliovirus would be absent only in very young Africans, and that almost 100% of children over 5 years of age would be seroimmune This prediction was confirmed, first by Pattyn, *et al*,[3] who tested 78 sera from Africans living in Leopoldville; and second, by tests of over 1,300 sera obtained throughout the city during the early stages of this vaccination campaign (August to early November, 1958)

The sera were tested at a 1 4 dilution in the metabolic inhibition test[4] The results are given in Table 1 and in Fig 1 The percentage of sera positive for Type 1 polio antibodies was 9% for children from 6 months to one year of age, 23% for those 1-2 years, 46% for those 2-3 years, 67% for those 3-4 years, and 90% for those 4-5 years old The percentage of children with

TABLE 1 ANTIBODIES TO POLIOMYELITIS IN SERA OF LEOPOLDVILLE AFRICAN CHILDREN

Age in Years	Antibodies to Polio Virus											
	Type 1			Type 2			Type 3			No Type*		
	No Tested	No Pos	% Pos	No Tested	No Pos	% Pos	No Tested	No Pos	% Pos	No Tested†	No Neg†	% Neg
½–1	411	35	9	350	23	7	375	64	17	340	251	74
1–2	615	139	23	553	71	13	579	213	37	536	248	46
2–3	199	92	46	172	55	31	188	95	51	165	29	18
3–4	52	35	67	45	24	53	48	29	60	44	3	7
4–5	41	37	90	25	24	96	26	21	81	24	0	0
Totals	1318	338	26	1145	197	17	1216	402	33	1109	531	48

* Triple negatives
† For all three types

5 Same specimens from post-vaccination "aseptic meningitis"

Records

1 Serially numbered health inquiry cards—10-20,000 Ship in one lot to Wistar unless suspicion indicates earlier shipment
2 Detailed bleeding record (x3).
3 Cumulative blood specimen summary by age
4 Daily work log of vaccinations, inquiries, bleeding
5 Detailed record of reported polio cases (3x)

Note Aliquots of vaccine can be sent frozen with specimens from patients, every 2 months or so, to Wistar for periodical testing

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. C. Dricot, Médecin en Chef, Directeur Général des Services médicaux du Congo Belge et du Ruanda-Urundi, and to Dr. W. P Bervoets Médecin Inspecteur des Services d'Hygiène, for their helpful advice and administrative cooperation during this trial. We also wish to acknowledge the hard work of Dr. Herman, Mlle H Kint, and Mme Page in the daily control of vaccination We acknowledge equally the aid received from Dr. Vandeputte, who kindly gave the figures obtained in the Virology section of I M T P A

Vaccination

In view of the observed age incidence of polio in Leopoldville[a] (Table 8) is was decided to limit vaccination to children under 5 years of age The numbers of such children as determined by the Medical Census are given by district of the city in Table 2. there was a total of approximately 76 200 African children eligible for vaccination in metropolitan Leopoldville, 40 000 of whom were 6 months to 3 years old as of April 30, 1959, when this review was made, and nearly 46 000 children had received CHAT virus, the number by district is shown in Table 2

Table 1, it is possible to estimate the numbers of vaccinated children 6 months to 5 years old who lacked antibodies to Type 1 polio and those who had no antibodies to any of the three types Inasmuch as no sera were obtained from infants under 6 months, it is not possible to estimate the total number who lacked antibody. As shown in Table 4, more than 21,800 Type 1 negatives were vaccinated, including at least 12,100 triple negatives

Safety of the Virus Post Vaccination Inquiries

In order to obtain data concerning the safety

TABLE 2. VACCINATION WITH CHAT VIRUS BY DISTRICT OF LEOPOLDVILLE TO APRIL 30, 1959

DISTRICT	TOTAL POPULATION (x 10³)	CHILDREN 6 MOS — 3 YRS (x 10³)	CHILDREN UNDER 5 YRS (x 10³)	NUMBER VACCINATED (UNDER 5 YRS)
Ancien Cité	125 8	13 5	24 8	19,182
Bandalungwa	13 2	2 1	3 9	1,735
Camp Leo	*	0.7	1 2	1,185
Kintambo	21 2	2 5	4 7	2,885
Matete	23 6	2 9	5 4	3,745
Ndjili	32 8	3 8	7 2	4,347
Nouvelle Cité	123 9	15 1	29 0	12,647
Totals		40 6	76 2	45,726

* Military encampment, figure not available

The cumulative totals of vaccinated individuals in each district are given in Table 3 It can be seen that the campaign was confined to the smaller districts of the city—Kintambo, Ndjili, Matete, Bandalungwa, and Camp Leopoldville—during the first nine weeks of vaccination Small numbers of children from the larger Ancienne and Nouvelle Cités were vaccinated at the end of October and the beginning of November, respectively, and larger numbers thereafter

Vaccination at well-baby clinics heavily weighted the age distribution of vaccinees with young infants, as shown in Table 4 The consequence of this is that more susceptibles were vaccinated than would have been the case if vaccinees had been selected at random

From the totals of vaccination presented in Table 3, and the serological data presented in

of the CHAT strain, a system of post-vaccination inquiries was formulated Two full time public health nurses were stationed at vaccination centers where approximately one of every five children was selected for follow-up. An index card was filled out for each child selected, bearing the name, address, age, and sex, as well as information concerning the health of the child during the week prior to vaccination

Eight days after vaccination, the same child was seen at home by a nurse, who also questioned the mother concerning the child's health during the previous week The information thus obtained was recorded on the card The same process was repeated 15 days after vaccination Later visits were made in some cases to ascertain the outcome of an illness found at 15 days

Type 2 and Type 3 polio antibodies increased similarly with age Type 3 antibodies, however, were found more often than Type 1 in children during the first three years of life, whereas Type 2 antibodies were found less frequently up to the age of 4-5 years The percentage of children with no antibodies to any of the three types of poliovirus, "triple negatives," decreased from 74% in those 6 months to one year of age to 0% at 4-5 years of age

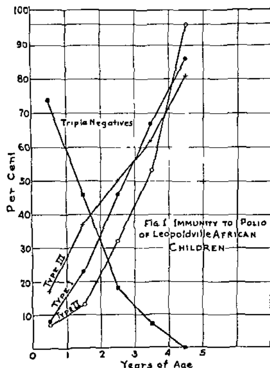


Fig 1 Seroimmunity to poliomyelitis of Leopoldville African children, as determined from the results of testing over 1300 sera

Thus the results showed that seroimmunity to poliomyelitis was acquired within the first five years of life by African children in Leopoldville, but that a large percentage of the infants under 3 years lacked antibodies

Geography and Demography of Leopoldville

In order to understand the data subsequently presented, some geographic orientation is necessary. Figure 2 is an outline map of metropolitan Leopoldville, showing its major subdivisions.

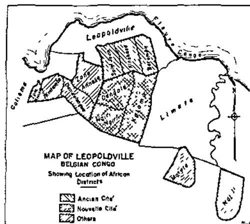


Fig 2 Map of Leopoldville, Belgian Congo, showing the location of African residential areas

The Leopoldville and Limete districts are inhabited by Europeans Saint Jean, Kinshasa, and Barumbu form the densely overcrowded "Ancien Cité," where a population of approximately 120,000 people live in huts and shacks with few sanitary facilities Dendale, Ngiri Ngiri, and Kalamu comprise the "Nouvelle Cité," also with a population of approximately 120,000 Although much of the housing in this section is in the form of European style single dwellings sanitary facilities still consist almost exclusively of pit privies There are 4 other districts plus 2 military zones in metropolitan Leopoldville, as shown on the map Kintambo has approximately 20,000 inhabitants and is an old section of the city, however, the housing is sturdier and better built than in the Ancien Cité The two Zone Annexes are the sites of military encampments, collectively referred to as Camp Leopoldville Bandalungwa, Matete, and Ndjili are recently built European style housing developments for Africans, with populations of 13,000, 26 000, and 36,000, respectively. In these areas, sanitation is accomplished by pit privies or by bored holes

It is important to note that the source of the demographic data is the Recensement Medicale —the Medical Census—which was mentioned by Dr. Lebrun The fact that each inhabitant must be examined yearly in order to stay within the law makes it possible to collect statistics which are estimated to include more than 95% of those actually living in the city.

Vaccination

In view of the observed age incidence of polio in Leopoldville* (Table 8) is was decided to limit vaccination to children under 5 years of age The numbers of such children as determined by the Medical Census are given by district of the city in Table 2. there was a total of approximately 76,200 African children eligible for vaccination in metropolitan Leopoldville, 40,000 of whom were 6 months to 3 years old as of April 30, 1959, when this review was made, and nearly 46,000 children had received CHAT virus, the number by district is shown in Table 2

Table 1, it is possible to estimate the numbers of vaccinated children 6 months to 5 years old who lacked antibodies to Type 1 polio and those who had no antibodies to any of the three types Inasmuch as no sera were obtained from infants under 6 months, it is not possible to estimate the total number who lacked antibody. As shown in Table 4, more than 21,800 Type 1 negatives were vaccinated, including at least 12,100 triple negatives

Safety of the Virus Post Vaccination Inquiries

In order to obtain data concerning the safety

TABLE 2 VACCINATION WITH CHAT VIRUS BY DISTRICT OF LEOPOLDVILLE TO APRIL 30, 1959

DISTRICT	TOTAL POPULATION (x 10³)	CHILDREN 6 MOS — 3 YRS (x 10³)	CHILDREN UNDER 5 YRS (x 10³)	NUMBER VACCINATED (UNDER 5 YRS)
Ancien Cite	125 8	13 5	24 8	19,182
Bandalungwa	13 2	2 1	3 9	1,735
Camp Leo	*	0 7	1 2	1,185
Kintambo	21 2	2 5	4 7	2,885
Matete	23 6	2 9	5 4	3,745
Ndjili	32 8	3 8	7 2	4 347
Nouvelle Cite	123 9	15 1	29 0	12,647
Totals		40 6	76 2	45,726

* Military encampment, figure not available

The cumulative totals of vaccinated individuals in each district are given in Table 3 It can be seen that the campaign was confined to the smaller districts of the city—Kintambo, Ndjili, Matete, Bandalungwa, and Camp Leopoldville—during the first nine weeks of vaccination Small numbers of children from the larger Ancienne and Nouvelle Cités were vaccinated at the end of October and the beginning of November, respectively, and larger numbers thereafter

Vaccination at well-baby clinics heavily weighted the age distribution of vaccinees with young infants, as shown in Table 4 The consequence of this is that more susceptibles were vaccinated than would have been the case if vaccinees had been selected at random

From the totals of vaccination presented in Table 3, and the serological data presented in

of the CHAT strain, a system of post vaccination inquiries was formulated Two full-time public health nurses were stationed at vaccination centers where approximately one of every five chil

tion concerning the health of the child during the week prior to vaccination

Eight days after vaccination, the same child was seen at home by a nurse, who also questioned the mother concerning the child s health during the previous week The information thus obtained was recorded on the card The same process was repeated 15 days after vaccination Later visits were made in some cases to ascertain the outcome of an illness found at 15 days

Type 2 and Type 3 polio antibodies increased similarly with age Type 3 antibodies, however, were found more often than Type 1 in children during the first three years of life, whereas Type 2 antibodies were found less frequently up to the age of 4-5 years The percentage of children with no antibodies to any of the three types of poliovirus, "triple negatives," decreased from 74% in those 6 months to one year of age to 0% at 4-5 years of age

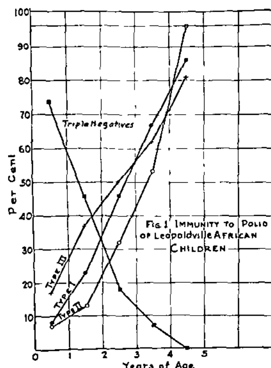


Fig 1. Seroimmunity to poliomyelitis of Leopoldville African children, as determined from the results of testing over 1300 sera

Thus the results showed that seroimmunity to poliomyelitis was acquired within the first five years of life by African children in Leopoldville, but that a large percentage of the infants under 3 years lacked antibodies.

Geography and Demography of Leopoldville

In order to understand the data subsequently presented, some geographic orientation is necessary. *Figure 2 is an outline map of metropolitan* Leopoldville, showing its major *subdivisions.*

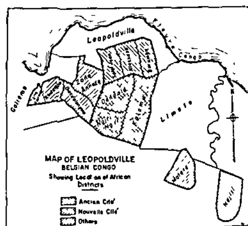


Fig 2 Map of Leopoldville, Belgian Congo, showing the location of African residential areas

The Leopoldville and Limete districts are inhabited by Europeans. Saint Jean, Kinshasa, and Barumbu form the densely overcrowded "Ancien Cité," where a population of approximately 120,000 people live in huts and shacks with few sanitary facilities Dendale, Ngiri Ngiri, and Kalamu comprise the "Nouvelle Cité," also with a population of approximately 120,000 Although much of the housing in this section is in the form of European style single dwellings, sanitary facilities still consist almost exclusively of pit privies There are 4 other districts plus 2 military zones in metropolitan Leopoldville, as shown on the map Kintambo has approximately 20,000 inhabitants and is an old section of the city, however, the housing is sturdier *and better* built than in the Ancien Cité The two Zone Annexes are the sites of military encampments, collectively referred to as Camp Leopoldville Bandalungwa, Matete, and Ndjili are recently built European style housing developments for Africans, with populations of 13,000, 26,000, and 36,000, respectively. In these areas, sanitation is accomplished by pit privies or by bored holes

It is important to note that the source of the demographic data is the Recensement Medicale —the Medical Census—which was mentioned by Dr. Lebrun The fact that each inhabitant must be examined yearly in order to stay within the law makes it possible to collect statistics which are estimated to include more than 95% of those *actually living in the city.*

TABLE 3—Continued

CUMULATIVE TOTALS OF VACCINATIONS BY DISTRICT (× 10³)

Week Beginning	Kintambo	Ndjili	Matete	Bandalungwa	Camp Léo	Ancien Cité	Nouvelle Cité	Total	Vaccination Centers* In Use
March 2	2.6	4.1	3.0	1.5	1.2	14.1	7.5	34.0	B,U,X,W,Y
9	2.6	4.1	3.1	1.6	1.2	14.9	8.2	35.7	U,V,W,X,Y
16	2.7	4.2	3.1	1.6	1.2	15.5	8.9	37.1	X,V,W,Y
23	2.7	4.2	3.1	1.6	1.2	16.4	9.5	38.7	X,V,Y
30	2.8	4.2	3.2	1.6	1.2	17.2	10.1	40.2	X,Y
April 6	2.8	4.2	3.2	1.6	1.2	17.5	10.4	41.0	Y
13	2.8	4.3	3.3	1.7	1.2	18.0	10.8	42.0	Y
20	2.8	4.3	3.5	1.7	1.2	18.7	11.9	44.0	Y
27	2.9	4.3	3.7	1.7	1.2	19.2	12.6	45.7	Y

* Locations shown in Figs 4, 7, and 8

TABLE 3 PROGRESS OF VACCINATION WITH CHAT VIRUS IN LEOPOLDVILLE

WEEK BEGINNING	CUMULATIVE TOTALS OF VACCINATIONS BY DISTRICT (× 10⁴)								VACCINATION CENTERS* IN USE
	KINTAMBO	NDJILI	MATETE	BANDA-LUNGWA	CAMP LÉO	ANCIEN CITÉ	NOUVELLE CITÉ	TOTAL	
1958									
Aug 18	15	—	—	—	—	—	—	15	A
25	19	05	—	—	—	—	—	25	C
Sept. 1	19	05	—	—	—	—	—	25	
8	19	35	08	—	—	—	—	63	C,D
13	19	35	24	—	—	—	—	79	D
22	19	35	24	10	—	—	—	89	E
29	19	35	24	10	—	—	—	89	
Oct 6	19	35	24	10	08	—	—	97	F
13	19	35	24	10	08	—	—	97	
20	19	35	24	10	08	02	—	100	G
27	19	35	24	10	08	04	—	102	H,I
Nov 3	19	36	24	10	08	10	01	110	I,J,K,Y
10	20	36	24	11	08	13	03	114	K,Y
17	20	36	24	11	08	16	04	120	K,Y
24	20	36	25	11	08	20	06	125	K,Y
Dec 1	20	37	25	11	08	27	08	13.7	L,M,N,Y
8	21	38	25	12	12	36	12	155	L,N,O,P,Y
15	21	38	26	12	12	45	15	169	L,N,Y
22	21	39	26	12	12	47	17	17.3	L,N,Y
29	21	39	26	12	12	50	20	180	R,Y
1959									
Jan 5	21	39	26	12	12	51	21	183	Y
12	22	39	26	13	12	54	22	187	Y
19	22	40	27	13	12	63	31	208	Y
26	23	40	28	14	12	66	34	216	S,Y
Feb 2	23	40	28	14	12	74	46	237	T,U,Y
9	23	40	29	14	12	88	53	259	S,T,U,Y
16	21	41	29	1.4	12	118	60	292	B,S,T,U,Y
23	25	41	30	15	12	127	67	316	B,T,U,X,Y

Table 3—Continued

Week Beginning	Cumulative Totals of Vaccinations by District (× 10³)								Vaccination Centers* In Use
	Kintambo	Ndjili	Matete	Banda-lungwa	Camp Léo	Ancien Cité	Nouvelle Cité	Total	
March 2	2 6	4 1	3 0	1 5	1 2	14 1	7 5	31 0	B,U,X,W,Y
9	2 6	4 1	3 1	1 6	1 2	14 9	8 2	35 7	U,V,W,X,Y
16	2 7	4 2	3 1	1 6	1 2	15 5	8 9	37 1	X,V,W,Y
23	2 7	4 2	3 1	1 6	1 2	16 4	9 5	38 7	X,V,Y
30	2 8	4 2	3 2	1 6	1 2	17 2	10 1	40 2	X,Y
April 6	2 8	4 2	3 2	1 6	1 2	17 5	10 4	41 0	Y
13	2 8	4 3	3 3	1 7	1 2	18 0	10 8	42 0	Y
20	2 8	4 3	3 5	1 7	1 2	18 7	11 9	44 0	Y
27	2 9	4 3	3 7	1 7	1 2	19 2	12 6	45 7	Y

* Locations shown in Figs 4, 7, and 8

Table 3 Progress of Vaccination with CHAT Virus in Leopoldville

Week Beginning	Cumulative Totals of Vaccinations by District (× 10³)								Vaccination Centers* In Use
	Kintambo	Ndjili	Matete	Banda-lungwa	Camp Léo	Ancien Cité	Nouvelle Cité	Total	
1958									
Aug 18	1 5	—	—	—	—	—	—	1 5	A
25	1 9	0 5	—	—	—	—	—	2 5	C
Sept 1	1 9	0 5	—	—	—	—	—	2 5	
8	1 9	3 5	0 8	—	—	—	—	6 3	C,D
13	1 9	3 5	2 4	—	—	—	—	7 9	D
22	1 9	3 5	2 4	1 0	—	—	—	8 9	E
29	1 9	3 5	2 4	1 0	—	—	—	8 9	
Oct 6	1 9	3 5	2 4	1 0	0 8	—	—	9 7	F
13	1 9	3 5	2 4	1 0	0 8	—	—	9 7	
20	1 9	3 5	2 4	1 0	0 8	0 2	—	10 0	G
27	1 9	3 5	2 4	1 0	0 8	0 4	—	10 2	H,I
Nov 3	1 9	3 6	2 4	1 0	0 8	1 0	0 1	11 0	I,J,K,Y
10	2 0	3 6	2 4	1 1	0 8	1.3	0 3	11.4	K,Y
17	2 0	3 6	2 4	1 1	0 8	1 6	0 4	12 0	K,Y
24	2 0	3 6	2 5	1 1	0 8	2 0	0 6	12 5	K,Y
Dec 1	2 0	3 7	2 5	1 1	0 8	2 7	0 8	13 7	L,M,N,Y
8	2 1	3 8	2 5	1 2	1 2	3 6	1 2	15 5	L,N,O,P,Y
15	2 1	3 8	2 6	1 2	1 2	4 5	1 5	16 9	L,N,Y
22	2 1	3 9	2 6	1 2	1 2	4 7	1 7	17 3	L,N,Y
29	2 1	3 9	2 6	1 2	1 2	5 0	2 0	18 0	R,Y
1959									
Jan 5	2 1	3 9	2 6	1 2	1 2	5 1	2 1	18 3	Y
12	2 2	3 9	2 6	1 3	1 2	5 4	2 2	18 7	Y
19	2 2	4 0	2 7	1 3	1 2	6 3	3 1	20 8	Y
26	2 3	4 0	2 8	1 4	1 2	6 6	3 4	21 6	S,Y
Feb 2	2 3	4 0	2 8	1 4	1 2	7 4	4 6	23 7	T,U,Y
9	2 3	4 0	2 9	1 4	1 2	8 8	5 3	25 9	S,T,U,Y
16	2 4	4 1	2 9	1 4	1 2	11 8	6 0	29 2	B,S,T,U,Y
23	2 5	4 1	3 0	1 5	1 2	12 7	6 7	31 6	B,T,U,X,Y

TABLE 6 ILLNESSES DISCOVERED BY POST-VACCINATION INQUIRIES

ILLNESS CATEGORY	DURING WEEK BEFORE VACCINATION N	% TOTAL	FIRST 8 DAYS AFTER VACCINATION N	% TOTAL	8–15 DAYS AFTER VACCINATION N	% TOTAL
Upper Resp	374	(5)	563	(8)	590	(8)
Digestive	126	(2)	208	(3)	213	(3)
Skin & Mucosal	39	(5)	70	(1)	51	(7)
Fever	14	(2)	54	(7)	32	(4)
Lower Resp	9	(1)	17	(2)	21	(3)
Anemia	19	(2)	25	(3)	21	(3)
Miscellaneous	8	(1)	31	(4)	39	(5)
No symptoms	7056	(92)	6343	(87)	6225	(87)
Totals	7645	(100)	7311	(100)	7195	(100)

$n = 14$ and $p = < 01$). The actual significance of this difference is doubtful, since the popula tion coming for vaccination was probably selected for good health. Children who were ill would *probably not have been brought for vaccination* if during the next 15 days these children suffered a usual amount of illness, an apparent increase in incidence would result As evidence for this explanation one can cite the incidences of illnesses presumably unrelated to vaccination, as, for example, "upper" and "lower respiratory," "skin and mucosal," and "anemia" It will be noted in Table 6 that the incidence of each of these types of illness increased by 50 to 100% over the pre vaccination figures This difference was also significant ($x^2 = 86\,6$, $n = 6$, $p = < 01$), which implies that the post vaccination and pre-vaccination inquiries were not comparable If this explanation is correct, one *would expect that the percentage of illness found* would be the same for both post-vaccination visits Illnesses caused by the virus would be expected to become evidence during the second post-vaccination week In fact, the incidence of illness is the same during the second week after ingestion of the virus as during the first

Another factor of importance in evaluating the significance of these data is the accuracy of the answers volunteered by the mothers It was the impression of the interviewers that minor illnesses were sometimes ignored and sometimes exaggerated Consequently, these figures are important not as evidence relative to the presence or absence of minor reactions to live-virus vaccination, but rather as an indication of the care with which the inquiries for major illnesses were conducted

It must be emphasized that the principal object of the inquiries was the detection of neurological *reactions to vaccination (listed under the category* of "Miscellaneous" in Table 6) Serious illnesses discovered on post-vaccination inquiry are analyzed in detail in Table 7 The single illness of note was a case of "encephalitis" in a four-year-old girl, who developed fever, profound somnolence, and paraplegia sixteen days after ingesting CHAT virus She was admitted to the Contagious Disease Hospital where a neurological consultant found spastic paralysis of both legs with positive Babinski signs and hyperreflexia A lumbar puncture revealed normal cerebrospinal fluid The consultant's diagnosis was acute encephalitis The child was also seen by the present author in May, 1959, eight months subsequent to the acute illness At this time she walked *with a* "scissors" gait and was without

TABLE 7 MISCELLANEOUS ILLNESSES DISCOVERED ON POST-VACCINATION INQUIRIES

Varicella	42
Rubeola	14
Mumps	3
Otitis	4
Malaria	3
Infection at Venipuncture Site	1
Retropharyngeal Abscess	1
Death from Pneumonia	1
Encephalitis	1

TABLE 4 AGE DISTRIBUTION AND SUSCEPTIBILITY OF VACCINATED POPULATION

Age in Years	Number Vaccinated	Estimated Number Type 1 Neg.* Vaccinated (x 10^3)	Estimated Number Triple Non-Immunes* Vaccinated (x 10^3)
<1	14,924	—	—
<½	7,600†	N.T.	N.T.
½–1	7,300†	6.7	5.4
1–2	10,340	8.0	4.8
2–3	7,827	4.2	1.4
3–4	7,027	2.3	0.5
4–5	5,608	0.6	—
Totals	45,726	21.8†	12.1†

* For serological percentages, see Table 1
† Estimated
N.T. = Not tested

Thus, 7,645 children were registered for follow-up, of which 7,311 were seen at 8 days post-vaccination, and 7,195 at 15 days post-vaccination. The difference between the first two figures is due to incorrect addresses, and the *difference between the second and third figures* is the result of families having moved.

The age distribution of completed post-vaccination inquiries, shown in Table 5, was markedly shifted towards young infants. 59% of the children about whom inquiries were made were less than one year of age. Using the serological data in Table 1 to partially estimate the number of susceptible individuals followed after vaccination, we find that more than 3,400 Type 1 negative and 2,100 triple negative children were checked by home visits after vaccination.

The illnesses reported to the nurses on the *health inquiries are analyzed in* Table 6 according to the class of illness. The largest number of illnesses were: (1) upper respiratory in nature, and (2) gastrointestinal complaints.

The total rate of illness reported was 8% in the week before vaccination and 13% in each of the two weeks following vaccination (χ^2 = 182.2 for seven classes of illness and 1 of health,

TABLE 5 POST-VACCINATION INQUIRIES BY AGE AND SEROLOGICAL STATUS

Age in Years	Number Inquiries Completed	Estimated Type 1 Neg.* (x 10^3)	Estimated Triple Non-Immunes* (x 10^3)
<1	2,980	—	—
<½	1,520†	N.T.	N.T.
½–1	1,460†	1.3	1.1
1–2	1,611	1.2	0.7
2–3	852	0.5	0.2
3–4	979	0.3	0.1
4–5	773	0.1	—
Totals	7,195	3.4†	2.1†

* For serological percentages, see Table 1
† Estimated
N.T. = Not tested

The age distribution of the paralytic cases is given in Table 9; as in the past, the majority of cases were in the 1-2 year-old age group, with over 95% under three years of age. The clinical localization of paralysis was also quite similar to that observed in the past, with involvement of the lower extremities only occurring in over 85% of cases (Table 10)

in children in the Nouvelle Cité In the 1958-59 epidemic, however, the ratio was reversed and the cases in the Ancien Cité numbered two-thirds of those in the Nouvelle Cite. A possible explanation for this reversal is offered later based on the protective effect of vaccination

The attack rate in Leopoldville for this epidemic was *28 6* per *100,000* (*99* cases in *346,000*

TABLE 9 1958-59 EPIDEMIC CASES OF POLIOMYELITIS ANALYZED BY AGE AND COMPARED TO PRIOR EXPERIENCE

AGE IN YEARS	1/56—7/58		8/58—4/59	
	N	%	N	%
<1	50	30	25	25
1–2	83	50	57	58
2–3	20	12	15	15
3–4	6	4	1	1
4–5	1	1	—	—
>5	6	4	1	1
Totals	166	101	99	100

TABLE 10. DISTRIBUTION OF PARALYSIS IN 1958-59 EPIDEMIC CASES COMPARED TO PREVIOUS YEARS

SITE OF PARALYSIS	1/56—7/58		8/58—4/59	
	N	%	N	%
One leg	79	56	52	54
Both legs	49	35	32	33
Arms & legs	9	6	10	10
One arm	2	1	—	—
Bulbar	2	1	2	2
Totals	141	99	96*	99

* Three additional cases without information

Examination of the geographic localization of the cases revealed a distinct difference between the 1958-1959 epidemic and the experience from 1951 up until the end of 1958 As shown in Table 11, the greatest number of cases in past years has occurred in children living in the Ancien Cité, with only two thirds as many cases

population) If, however, only children under five are considered, the incidence becomes 129 per 100,000 (98/76 200) The peak age specific incidence was in the 1 2 year-olds, where 344 per 105,000 (57 16,600) were affected If only Type 1 susceptibles are considered, the attack rate in this age group was an astonishing 445 per

evidence of muscular weakness or atrophy. Hyperreflexia persisted, and the child had difficulty in speech. It was concluded that this illness was not caused by poliomyelitis infection

With regard to safety, therefore, it may be said that in 7,200 children who were fed CHAT virus and who were observed directly for reactions to vaccination, there was no clinically recognizable poliomyelitis Of this number, at least 3,400 were Type 1 susceptible In evaluating proof of safety, the figures must be corrected for the percentage of individuals who showed serological evidence of having been infected by the attenuated virus, a point which is discussed below

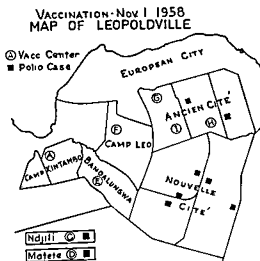


Fig. 3 Map of Leopoldville showing the location of cases of paralytic poliomyelitis occurring between the beginning of the vaccination campaign on August 18, 1958 and November 1, 1959 Also shown are the sites of vaccination used during that period The numbers vaccinated at each center and the dates of vaccination are given in Table 3

Surveillance of Poliomyelitis in Leopoldville During Vaccination

As shown by Dr. Lebrun,* poliomyelitis is both endemic and epidemic in Leopoldville and other parts of the Belgian Congo. In Table 8 are given the reported number of cases from the city and province of Leopoldville during the eight months of 1958 just prior to the vaccination campaign In the city, 23 cases occurred during January to March, 1958, which represented the end of an epidemic that had begun in November, 1957 In Leopoldville province, 41 cases of paralytic poliomyelitis were reported from June to August, 1958, mostly from the town of Tshela Type 1 polio virus was isolated from the stools of some of these cases, and serological studies showed recent activity of this agent

An epidemic of paralytic poliomyelitis due to Type 1 virus broke out in Leopoldville city at the end of October, 1958, two months after the commencement of vaccination. The epidemic curve (Fig 4) shows a peak in December, 1958, and ends in March, 1959 There were 99 cases of poliomyelitis, including 4 deaths

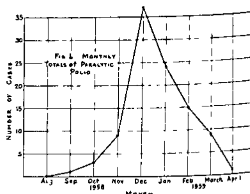


Fig. 4 Monthly totals of cases of paralytic poliomyelitis occurring among Africans in Leopoldville from August, 1958 to April, 1959

Table 8 Reported Poliomyelitis Cases by Month—Leopoldville City and Province January-August, 1958

	Jan	Feb	Mar	April	May	June	July	Aug
City	12	5	6	1	2	—	1	—
Province	7	3	4	—	3	10	22	9

TABLE 13. PARALYTIC POLIOMYELITIS IN PREVIOUSLY VACCINATED INDIVIDUALS

CASE NO	AGE	DATE OF VACCINATION (1958–59)	DATE OF ONSET OF ILLNESS (1958–59)	INTERVAL VACC TO ONSET	EXTENT OF PARALYSIS	LABORATORY DATA*
14B	2 y	Aug 18	Nov 20	3 m	Rt leg	CF Test†—Type 1 Polio
62	1 y	Sept 20–30	Jan 6	3 m	Rt. leg	
63	1 y	Jan 9 or 10	Jan 11	1–2 d	Both legs	Stool—Type 1 Polio
77	20 m	Sept 22–27	Jan 24	4 m.	Left leg	CF & Stool—Type 1 Polio
81	15 m	Feb 2	Feb 8(?)	6 d (or <)	Left leg	Stool—Type 1 Polio
87	9 m	Dec 5	Feb 6	2 m	All 4 limbs	CF & Stool—Type 1 Polio
92	15 m	Dec 5	Feb 25	2½ m	Rt leg	
93	1 y	Feb 6	Feb 26	20 d	Left leg	CF & Stool—Type 1 Polio
99	11 m	Jan 17	Mar 14	2 m	Left leg	Stool—Type 1 Polio
101	2 y	Jan 21	Mar 22	2 m	Legs & arm	

* Stools and blood obtained within 1 week of date of onset
† Complement Fixation Test

positive complement fixation test for Type 1 virus Although the positive complement fixation test may conceivably have been due to attenuated virus administration 3 months before, it is perhaps more likely that this child was one of those who failed to react to the vaccine, and who therefore remained susceptible (see below)

The interval between vaccination and the onset of illness was two months or more in 7 of the 10 vaccinated individuals In case No 63, illness began one or two days after the administration of CHAT, in case No 81, illness is said to have *begun 6 days after vaccination, however, when* the child was seen 8 days post vaccination, she was already afebrile, suggesting the possibility that illness may have begun in actuality at an earlier date than February 6 The tenth case (No. 93) occurred 20 days after vaccination This figure is the minimal interval which could be derived from the history and is perhaps one or two days less than the actual interval

In relation to the safety of the vaccine to the vaccinees, cases No 81 and No 93 require consideration *Dr Koprowski's paper,*[*] *has pre*sented evidence that the strains isolated from these two patients were clearly distinguishable from the CHAT strain by serological and other genetic markers, and bore the characteristics of the wild virulent viruses isolated from nonvaccinated paralytic cases during the epidemic in Leopoldville

Evolution of the Epidemic

In the discussions which follow, the cases of poliomyelitis are dated as of their probable date of infection, which was calculated by antedating the day of reporting by 14 days The justification for this procedure was 1) that in the 24 cases where the date of onset was definitely known, the interval between onset and reporting averaged 7 days with a standard deviation of ± 5 days, and 2) added to this was an assumed incubation period of 7 days from the time of infection *to the time of overt signs of illness* *

The progress of the vaccination campaign in the various quarters of Leopoldville has been given in detail in Table 3 and the weekly totals of poliomyelitis cases during the vaccination campaign are shown in Table 14 The first evidence of an epidemic of poliomyelitis in Leopold ville came at the end of October The epidemiological situation as of November 1, 1958, is depicted in Fig 4 This date is of importance because prior to November 3, no vaccine had been given in the Nouvelle Cité, and only 433 children had been vaccinated in the Ancien Cite (Figs 5 and 6) The first case of poliomyelitis after the commencement of vaccination in Leopoldville was infected in the Nouvelle Cité dur

TABLE 11. LOCATION OF CASES OF POLIO BY DISTRICT OF LEOPOLDVILLE 1951-1958

DISTRICT	YEAR								
	1951	1952	1953	1954	1955	1956	1957	1958*	1958–1959†
Ancien Cité	25	35	30	8	39	30	27	9	33
Nouvelle Cité	15	20	27	5	17	22	22	6	49
Camp Léo	0	2	2	—	2	4	1	—	1
Kintambo	3	2	—	—	6	4	5	3	2
Matete‡	—	—	—	1	6	5	2	3	4
Ndjili‡	—	—	—	—	2	9	3	1	4
Bandalungwa‡	—	—	—	—	—	1	2	2	6
Unknown	2	6	2	1	2	3	3	3	—

* Jan-July
† Aug 1958—Apr 1959.
‡ New communities first inhabited in 1954-56

105,000 (57, 12,800), a figure which does not include the possible protective effect of vaccination

In order to confirm the diagnosis of paralytic polio, one of the present authors (S A P.) examined 55 of the cases of poliomyelitis reported during the epidemic In 50 of the patients, there was unequivocal flaccid paralysis or paresis, usually accompanied by muscular atrophy and hyporeflexia In 4 instances, no such changes were found Inasmuch as the examinations were conducted several months after the acute illnesses, and were gross rather than detailed muscle tests, these cases may well have been poliomyelitis In only one case it was our impression that the illness was unrelated to polio (brachial plexus injury?) In addition, 29 hospital records were checked, and all seemed consistent with poliomyelitis It is believed that the clinical diagnosis of paralytic poliomyelitis in Leopoldville is comparable in accuracy to other large cities

The laboratory study of this epidemic was discussed by Dr. Koprowski[5] It need only be pointed out here that the epidemic was proven by virus isolation and serological response to be due to Type 1 polio virus

TABLE 12 VACCINATION STATUS OF 1958-59 LEOPOLDVILLE EPIDEMIC POLIOMYELITIS CASES

DISTRICT	VACCINATED	NON-VACCINATED
Nouvelle Cite	2*	47
Ancien Cité	4	29
Bandalungwa	2	4
Ndjili	1	3
Matete	—	4
Kintambo	1	1
Camp Leo	—	1
Totals	10	89

* Cases Nos 63 and 81 in Table 13, which may have been in incubation period when vaccinated

Poliomyelitis in Vaccinated Individuals

Of the 99 epidemic cases, 89 occurred in non vaccinated and 10 in vaccinated individuals The predominance of cases in non-vaccinated children was true in all geographical subdivisions of Leopoldville (Table 12) The details of the 10 vaccinated cases are given in Table 13, from which it can be seen that the age distribution and clinical characteristics of the cases were roughly comparable to those found in the non-vaccinated cases.

Type 1 polio virus was isolated from the stools of all 6 patients from whom specimens were obtained. A seventh patient (No. 14B) had a

TABLE 14 DATES OF PROBABLE INFECTION OF LEOPOLDVILLE PARALYTIC POLIOMYELITIS CASES, BY WEEK—AUGUST 18, 1958-APRIL 30, 1959

	DISTRICT							
WEEKS BEGINNING	ANCIEN CITÉ	NOUVELLE CITÉ	BANDA-LUNGWA	NDJILI	MATETE	KINTAMBO	CAMP LÉO	TOTALS
Aug 18,25	—	—	—	—	—	—	—	—
Sept 1	—	1	—	—	—	—	—	1
8,15,22	—	—	—	—	—	—	—	—
29	—	—	—	—	1	—	—	1
Oct. 6	—	—	—	—	—	—	—	—
13	1	1	—	—	—	—	—	2
20	—	1	—	1	—	—	—	2
27	1	2	—	—	—	—	—	3
Nov 3	1	1	1	—	—	1*	—	4
10	—	1	—	—	—	—	—	1
17	2	5	—	—	—	—	1	8
24	1	6	—	—	—	—	—	7
Dec 1	4†	4	—	1	—	—	—	9
8	—	6†	1	—	—	—	—	7
15	2	6	—	1	—	—	—	9
22	3	2	—	—	—	—	—	5
29	2	1*	1*	—	—	—	—	4
Jan 5	1	3	1	—	—	1	—	6
12	3	1	1*	—	—	—	—	5
19	—	3	—	—	—	—	—	3
26	3	1*	—	—	1	—	—	5
Feb 2	2	—	1	1*	—	—	—	4
9	2	1	—	—	—	—	—	3
16	2‡	—	—	—	—	—	—	2
23	—	1	—	—	2	—	—	3
Mar 2	2*	1	—	—	—	—	—	3
9	1*	—	—	—	—	—	—	1
16,23	—	—	—	—	—	—	—	—
30	—	1	—	—	—	—	—	1
Apr 6–30	—	—	—	—	—	—	—	—

* One vaccinated case

† One case in a child older than 3 years.

‡ Two vaccinated cases.

37 more cases occurred, distributed throughout Leopoldville

The geographical locations of all of the epidemic cases and of the vaccination centers are depicted in Fig 8 Careful inspection of this figure, with reference to Table 3, for the dates on which vaccination was done, will reveal no clustering of cases around vaccination sites The apparent clusters near centers S and U disappear when it is realized that most of the cases had their onsets before vaccination was performed at those centers in February and March (Table 3)

The fact that an epidemic followed the use of living virus requires careful evaluation. It has been shown that in the two quarters in which

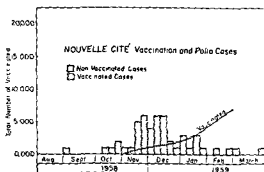


FIG. 5. The cumulative total of vaccinated individuals in the Nouvelle Cité district is indicated against the background of the cases of paralytic poliomyelitis which occurred during the same period. The week on which a case is placed is the week of presumed infection (see text)

ing the first week in September There was no known contact between this child and a vaccinated subject from another district The second paralyzed child lived in Matete (Fig. 2), and developed infection 4 weeks after vaccination had started in that district A week before the first vaccination in the Ancien Cité (October 20) the third case became infected Eight weeks after the first CHAT virus had been administered in Ndjili (Fig 2), a child living in that district became the fourth Leopoldville case In the same week, another case developed in the Nouvelle Cité, followed by three more during the following two-week period (October 20-November 1)

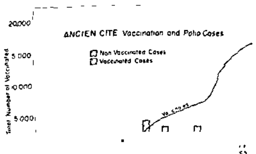


FIG. 6 The cumulative total of vaccinated ... occurred during the same period The week on which a case is placed is the week of presumed infection (see text)

A second case in the Ancien Cité was apparently infected during the second week of vaccination in that district, when only 433 children had received vaccine. This paralyzed child lived 06 km. from the nearest vaccination center Thus, none of the 9 cases of poliomyelitis occurring up to November 1 were vaccinated, and 7 of the 9 were unlikely to have had contact with CHAT virus As shown graphically in Figs 5 and 6, paralytic poliomyelitis appeared in the Nouvelle Cité and the Ancien Cité prior to the use of vaccine in these districts.

The subsequent development of the epidemic is shown in Fig. 7, which is an epidemiological map of Leopoldville as of January 1, 1959. In comparison to the situation at the beginning of November (Fig. 4) it can be seen that the epidemic had proceeded principally in the Nouvelle Cité The totals for the epidemic at the beginning of the year was 37 cases in the Nouvelle Cité, 17 in the Ancien Cité, and 8 elsewhere in Leopoldville During January to March, 1959,

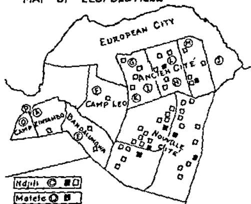


FIG 7. Map of Leopoldville showing the location of cases of paralytic poliomyelitis occurring between the beginning of the vaccination campaign on August 18, 1958 and January 1, 1959 The cases shown on Fig 4 are represented by black squares, whereas cases occurring since November 1 are represented by white squares. Also shown are the sites of vaccination used during that period The numbers vaccinated at each center and the dates of vaccination are given in Table 3

individuals provides no positive epidemiological evidence for a relationship between the vaccine virus and the epidemic The laboratory differentiation of "wild" virulent virus from attenuated virus after multiple human intestinal passages has already been discussed [5]

Efficacy of Vaccination Production of Antibodies

Nearly 400 sera from individuals lacking Type 1 antibodies before vaccination were collected in November, 1958 to January, 1959 The results of testing these sera (Table 16) reveal that only 60% developed Type 1 neutralizing antibodies after CHAT virus administration Approximately 100 sera tested for development of heterotypic polio antibodies showed 7% conversion each for Type 2 and Type 3 Thirty-five per cent of 62 non-vaccinated individuals also developed Type 1 antibodies during the same interval

The low serologic response was surprising considering the results obtained with the CHAT strain both in small, carefully controlled groups [6] and in field studies by Przesmycki *et al*,[9] using exactly the same pool of virus material as was used in Leopoldville These studies indicated a serological efficacy of 90 to 95% for the CHAT strain

The explanation for this disparity in results is obscure the most attractive hypothesis is based on the interfering effect of wild enteric viruses on live virus vaccinations as shown by Sabin,[10] Melnick,[11] and Paul *et al*,[12] earlier in this conference Some evidence for this opinion is found in Table 15 of the previous paper,[4] which shows that approximately 30% of asymptomatic children under 18 months of age excreted enteric virus detectable by lesions in HeLa tissue culture cells or baby mice.

TABLE 16 SEROLOGICAL CONVERSION AFTER CHAT VIRUS VACCINATION (ALL SERA NEGATIVE FOR TYPE INDICATED BEFORE VACCINATION)

Age in Years	Polio Type 1		Polio Type 2		Polio Type 3	
	No Tested	% Pos Conver	No Tested	% Pos Conver	No Tested	% Pos Conver
Vaccinated						
<1	105	56	30	13	29	0
1–2	182	64	54	2	52	6
2–3	45	56	9	22	11	27
3–4	8	63	2	0	2	50
Totals	340	60	95	7	94	7
Non-Vaccinated						
½–1	38	32	24	13	25	4
1–2	24	42	11	9	12	8
Totals	62	35	35	11	37	5

The 35% rate of conversion for Type 1 polio antibodies found in non-vaccinated individuals may be, of course, either the result of infection by the wild virulent virus or by inter-familial spread of the attenuated strain In the latter event, it is difficult to explain the low rate of conversion in the vaccinees, many of whom were in contact with vaccinated siblings While both mechanisms may have been operative, infection by wild virus seems more likely as the cause of antibody production in non vaccinated children, considering its wide dissemination in Leopoldville

Efficacy Protection Against Poliomyelitis

The circumstance of a polio epidemic in Leopoldville provided us with an opportunity to esti-

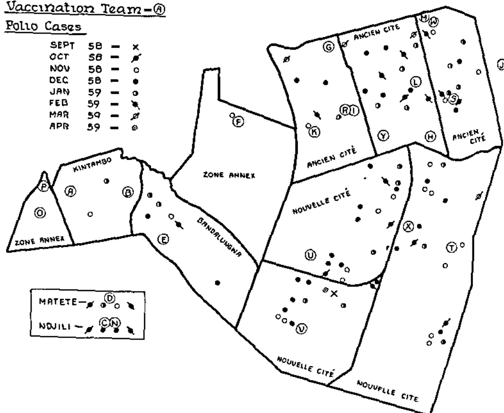


FIG 8 The locations of all of the cases of paralytic poliomyelitis which occurred in Leopoldville during the 1958 59 epidemic, and the sites at which vaccine was given If Table 3 is consulted for the numbers of vaccinated and the dates of vaccination at each center, a lack of relationship between cases of poliomyelitis and vaccine administration will be seen

poliomyelitis was most rampant, the Nouvelle Cité and the Ancien Cité, the first cases preceded vaccination, and the later cases were not related to the sites of vaccination

A fact of interest is the frequency of intimate contact of subsequently paralyzed children with vaccinees For 66 of the epidemic cases (61 non-vaccinated and 5 vaccinated) information was available as to the vaccination status of siblings As seen in Table 15, in only 4 of the 66 cases (2 vaccinated and 2 non-vaccinated) or 6%, were there vaccinated siblings in the family In view of the widespread vaccination, a 6% history of contact seems well within the province of chance

TABLE 15 VACCINATION HISTORY OF SIBLINGS OF LEOPOLDVILLE POLIO CASES

Vaccination Status of Polio Case	Vaccination Status of Siblings	
	Vaccinated	Non-Vaccinated
Vaccinated	2	3
Non-Vaccinated	2	59
Both	4	62

Thus, the lack of chronological or geographical association of cases and the lack of their association with vaccination centers or with vaccinated

individuals provides no positive epidemiological *evidence for a relationship between the vaccine* virus and the epidemic. The laboratory differentiation of "wild" virulent virus from attenuated virus after multiple human intestinal passages has already been discussed.[8]

Efficacy of Vaccination: Production of Antibodies

Nearly 400 sera from individuals lacking Type 1 antibodies before vaccination were collected in November, 1958 to January, 1959. The results of testing these sera (Table 16) reveal that only

The explanation for this disparity in results is obscure: *the most attractive hypothesis is based* on the interfering effect of wild enteric viruses on live virus vaccinations as shown by Sabin,[20] Melnick,[11] and Paul *et al*,[12] earlier in this conference. Some evidence for this opinion is found in Table 15 of the previous paper,[8] which shows that approximately 30% of asymptomatic children under 18 months of age excreted enteric virus detectable by lesions in HeLa tissue culture cells or baby mice.

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	No. TESTED	% POS. CONVER.	No. TESTED	% POS. CONVER.	No. TESTED	% POS. CONVER.
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<1	105	56	30	13	29	0
1–2	182	64	54	2	52	6
2–3	45	56	9	22	11	27
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Totals	340	60	95	7	94	7
Non-Vaccinated						
½–1	38	32	24	13	25	4
1–2	24	42	11	9	12	8
Totals	62	35	35	11	37	5

60% developed Type 1 neutralizing antibodies after CHAT virus administration. Approximately 100 sera tested for development of heterotypic polio antibodies showed 7% conversion each for Type 2 and Type 3. Thirty-five per cent of 62 non-vaccinated individuals also developed Type 1 antibodies during the same interval.

The low serologic response was surprising considering the results obtained with the CHAT strain both in small, carefully controlled groups[6] and in field studies by Przesmycki *et al*,[9] using exactly the same pool of virus material as was used in Leopoldville. These studies indicated a serological efficacy of 90 to 95% for the CHAT strain.

The 35% rate of conversion for Type 1 polio antibodies found in non-vaccinated individuals may be, of course, either the result of infection by the *wild virulent virus or by inter-familial* spread of the attenuated strain. In the latter event it is difficult to explain the low rate of conversion in the vaccinees, many of whom were in contact with vaccinated siblings. While both mechanisms may have been operative, infection by wild virus seems more likely as the cause of antibody production in non-vaccinated children, considering its wide *dissemination in Leopoldville*.

Efficacy: Protection Against Poliomyelitis

The circumstance of a polio epidemic in Leopoldville provided us with an opportunity to esti-

mate the protection afforded by attenuated virus

Although aware of the difficulties in measuring attack rates in a situation where part of the population is constantly changing from non-vaccinated to vaccinated, we felt that this opportunity should be utilized as fully as possible

Because all but 2 of the polio cases were in children under the age of 3 years, the evaluation of effectiveness was confined to this group

From inspection of the data in Table 3, it was clear that Leopoldville could be separated into three distinct divisions with respect to the interval between vaccination and the peak of the epidemic In the Nouvelle Cité the epidemic was largely over before vaccination commenced on a large scale; in the Ancien Cité the epidemic occurred *pari passu* with vaccination; and in the five smaller districts a considerable amount of vaccination was done largely prior to the epidemic. Accordingly, these three divisions were evaluated separately.

In the method employed to calculate attack rates for vaccinated or non-vaccinated populations, the numerator was the number of cases in the 6 months to 3 year-old group occurring from the commencement of vaccination in the particular district of Leopoldville to the end of March, 1959 (Table 14); and as the denominator the number of person-weeks exposure for the period from the first case occurring after vac-

TABLE 17 CUMULATIVE NUMBERS OF VACCINATED CHILDREN 6 MONTHS TO 3 YEARS OLD, BY WEEK ($\times 10^3$)

Week Beginning	District		
	Ancien Cité	Nouvelle Cité	Five Smaller Districts
1958			
Sept 22	—	—	4 9
29	—	—	4 9
Oct 6	—	—	5 4
13	—	—	5 4
20	0 1	—	5 4
27	0 2	—	5 4
Nov 3	0 6	0 1	5 5
10	0 7	0 1	5 5
17	0 9	0 2	5 5
24	1 1	0 3	5 6
Dec 1	1 5	0 5	5 6
8	2 0	0 7	6 0
15	2 5	0 8	6 1
22	2 6	0 9	6 1
29	2 8	1 1	6 1
1959			
Jan 5	2 9	1 1	6 2
12	3 0	1 2	6 2
19	3 5	1 7	6 4
26	3 7	1 9	6 4
Feb 2	4 1	2 6	6 5
9	4 9	2 9	6 5
16	6 6	3 3	6 7
23	7 1	3 7	6 8
Mar 2	7 8	4 2	7 0
9	8 3	4 6	7 0
16	8 7	5 0	7 2
23	9 2	5 3	7 2
30	9 6	5 6	7 4

cination to the last case For the calculation of person weeks, the weekly totals of susceptibles vaccinated were utilized (Table 17) The non-vaccinated exposure was obtained by subtracting the vaccinated person-weeks from the total number of person-weeks.

For example, in the Ancien Cite there were 4 vaccinated and 27 unvaccinated cases in children in the susceptible age group between October 27 and March 15 The number of susceptibles vaccinated in that district rose from 200 to 8,300 during the 20-week interval (Table 17) There were 64 800 vaccinated person-weeks of experience in that period Inasmuch as there was a total of 13,500 children 6 months to 3 years old in the Ancien Cité (Table 2), there were 205,200 unvaccinated person-weeks (270,000 — 64,800).

The summary results are given in Table 18, from which one can estimate a percentage protection against paralytic poliomyelitis of 53% in the Ancien Cité, 71% in the Nouvelle Cité, and 68% in the "five other districts" If we exclude

this epidemiological problem. Dr S Gard will present another method later in this conference.[13]

Conclusions

In summary, we have presented the preliminary results of trial of the CHAT strain of attenuated polio virus in Leopoldville, Belgian Congo. We conclude that

(1) The CHAT strain was tested in a community where there are excellent public health and hospital facilities and where poliomyelitis is both endemic and epidemic.

(2) Over 2 800 Type 1 susceptible African children less than five years old, of whom at least 1,260 were triple negative, were given the CHAT strain and followed after vaccination by home visits None of these children developed paralytic poliomyelitis or aseptic meningitis On the basis of the presence of antibody after vaccination, 60% of these children (or about 2,000), may have been infected successfully at vaccination.

(3) CHAT virus was administered to 46,000

TABLE 18 ESTIMATED PROTECTION OF CHAT-VIRUS AGAINST PARALYTIC POLIOMYELITIS: LEOPOLDVILLE AFRICAN CHILDREN 6 MONTHS TO 3 YEARS OF AGE

DISTRICT	GROUP	CASES*	PERSON-WEEKS*	RATE PER 10⁵	ESTIMATED PROTECTION
Ancien Cite	Vaccinated	4	64,800	6 2	53%
	Non-vaccinated	27	205,200	13 2	—
Nouvelle Cite	Vaccinated	2	47,800	4 2	71%
	Non-vaccinated	41	284,200	14 4	—
Five Other Districts	Vaccinated	4	130,400	3 0	68%
	Non-vaccinated	13	133,600	9 7	—

* For Ancien Cite Oct 27-March 15
For Nouvelle Cite Nov 3-April 5
For Five Other Districts Sept. 29-Feb 23

the Nouvelle Cite because vaccination largely followed the epidemic, an estimated protection of 60% is obtained

In this connection, it is important to note that only 60% of vaccinees developed Type 1 antibodies, so that the 10 cases in vaccinees may have been in those actually uninfected by CHAT virus Moreover, 2 of the 10 cases were probably in the incubation period when vaccinated

It is to be emphasized that there are other methods than the one given above for handling

children as of April 30, 1959 An estimated 21,800 were Type 1 *negative and 12,100 were* triple negative It was estimated that 13 200 of the 21,800 susceptibles were infected with CHAT virus As determined by the intervals between vaccination and the onset of illnesses, and by the laboratory study of the causative viruses, there were no reported cases of paralytic polio as a result of vaccination. Because of the excellent arrangement of public health and hospital services in Leopoldville, it is considered unlikely,

although no impossible, that a case of paralytic poliomyelitis might have been unreported

(4) Approximately two months after the beginning of vaccination, an epidemic of Type 1 poliomyelitis broke out in the African population The epidemic, although severe, was identical to previous outbreaks with respect to season of the year, age distribution, and types of paralytic involvement. The current epidemic was singular, however, in its concentration in one section of the city No vaccination had been done in this district prior to the beginning of the epidemic. No geographical, chronological, or familial association of vaccination and poliomyelitis cases was found Polio virus recovered from non-vaccinated cases differed in several characteristics from attenuated virus.

(5) Of the 99 cases of poliomyelitis, 89 developed in non-vaccinated individuals and 10 in vaccinated individuals Previous vaccination with CHAT virus appeared by one method of calculation to confer protection against the epidemic Type 1 poliovirus. The extent of protection was estimated to be 60% in the presence of a 60% seroconversion

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DISCUSSION

CHAIRMAN GEAR The papers presented by Dr Lebrun and by Dr Plotkin are open for discussion

Dr Courtois, your name appears on the program. Would you like to say anything?

DR. G COURTOIS *(through an interpreter)*: I should like to say a few words about polio vaccine during epidemic periods

When we started vaccinations in various cities, our paralytic cases took place earlier The results published earlier in the British Medical Journal were obtained from four foci caused by *virus No 1, and these make it difficult to deny* the value of this vaccination when it is applied under very difficult conditions and to deny the direct effect of the vaccination in the controlling and disappearance of the epidemic

The studies that we are doing now will make it possible to interpret the mechanism of these results Whether it is a question of the disappearance of the wild strain, with a substitution of an attenuated strain, or whether it is a *question of the insufficiency or absence of an* adequate quantity of receptive subjects who can be generally immunized, I believe that we can no longer call it coincidental or accidental, since this situation has been repeated four times, and we hope that it will be repeated again

There will certainly be skeptics, but at this time, when we have already seen four foci stopped in eight days, and we saw that no more cases arose, we cannot deny that there is a relationship *between the two*

DR PAUL We have had presented to us today, and yesterday, various types of field trials, small and large, and from them we have learned, I hope, that there are a number of methods of making observations and estimating what has happened

The small trials probably do not need much more discussion. We have heard the various ways in which they have been followed The larger trials are infinitely more difficult, and if anything can come from this Conference, among the many things that might be learned, some type of recognized technique or recognized general method of how to evaluate these studies, such as the one that Dr Plotkin has described, is a worthy objective. For instance, I am trying to decide whether, by the time the Conference has ended, any single case of paralytic polio will be presented which can be said to be an induced case due to the vaccine, or vice versa. How can anyone prove this with the methods we now have?

Dr Koprowski has spoken before, in this connection, of the use of the McBride test, which may be one very interesting point However, *over and above the remote chances of an induced* case, I think that before conclusions are reached as to exactly what happened in a field trial of the size presented, standard methods of surveillance, with which we are going to be in agreement, should be adopted

If that can come out of this Conference, then I think we shall have accomplished more than we had even hoped

DR HAMMON. The data presented to us with respect to the outbreak in Leopoldville, which occurred at about the same time, beginning apparently almost simultaneously with—possibly just before the vaccination and during the vaccination—are most interesting They require a great deal of careful study All of us ought to think about these data before we come to any firm conclusions, particularly with respect to the conclusion drawn by Dr. Plotkin that the vaccine *actually was effective in preventing the disease*

I believe that we still must have a trial with placebo controls if we are going to draw any really valid conclusions within a brief time period Such tests certainly can be carried out They are difficult, it is difficult to anticipate where one is going to have an outbreak and to start the test at the right time.

It is not as easy to do as with gamma globulin, which does not require time for active immunity *to develop; but if we are going* to evaluate this on the basis of incidence in vaccinated and non-vaccinated, I think it is going to have to be done

on a controlled placebo basis and not on the basis of such data as these

I think Dr Plotkin has pointed out some of the difficulties in interpreting the problem There is at least one additional factor Although he calculated "weeks of exposure" for the vaccinated and non-vaccinated, these "person-week exposures" were not always at the same level of risk as time progressed

The risk of each of those persons was changing, as one drew near the end of the epidemic and the number of vaccinated was greatest, and therefore the largest number of vaccinated persons was accumulating "weeks of exposure," the risk of exposure was rapidly decreasing The risk was greatest earlier when there were fewer "weeks of exposure" of vaccinated individuals

Therefore, in addition to adding certain other factors exerting an opposite influence on calculations, one must subtract a great deal for this factor of changing risk, that changing risk being in the opposite direction from the accumulation of "weeks of exposure"

This factor is most difficult to compensate for, and certainly, I think, it has been a big factor here.

I would be unwilling to say that I think there *was protection or was no protection* I just do not see how we can evaluate it

Dr Anderson Some of the remarks I was going to make when I first signalled have been made by Dr Hammon I do want to emphasize the importance, in this type of community studies, of calculating exposure on the basis of person-weeks, which Dr Plotkin has very nicely done. He should be commended on that, and we should urge that other investigators working on a community basis try to get some sort of an evaluation of degrees of exposure in terms of *person-time units.*

As I listened to the report yesterday from Singapore and tried to study through the data last evening, I felt that I could not make any evaluation because of the lack of information as to the person weeks of exposure, because we did not know at what rate the feeding was being conducted.

I hope that when Dr. Plotkin presents and publishes his data he will include a table showing the rate at which vaccination was going on in several sections of the city by weeks, so that we may make our own independent calculation—not that I do not trust his calculations, but I do think we should have the basic data in front of us

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We usually feel that when one enters into an epidemic situation and attempts to apply prophylactic measures, the epidemic is about *ready to start downhill, so that little can be said* about the effect of the immunizing agent. This has been shown again and again in the past.

What we have here is an instance where by chance, perhaps, the immunization program was begun and was immediately followed by an epidemic.

This is one of the most sobering bits of data that have come out of the entire Conference—that is, that with the immunization of the most susceptible segment of the population and with thoroughly high rates of immunization—from 42 to 100 per cent in the several districts—one *of the largest epidemics (quoting Dr. Lebrun),* or the largest in ten years, has started and gone through its normal course unaffected by what would have seemed to have been an ideal situation in terms of prevention

Dr Barr: Someone mentioned a placebo in the midst of this. *I would like to see what* manner of man, in the middle of an epidemic, would use placebos for vaccine, if he is convinced that the vaccine had any benefit

Dr Hammon: I have to answer this, I think, with a great deal of caution

To begin with, when we conduct an experiment, I do not think that we necessarily know ahead of time whether the agent we are testing is going to protect or not; otherwise, we would not do the experiment.

Therefore, I think that one would be justified, from the viewpoint of using a placebo, that he was not necessarily depriving people of something that was known to be effective. For at the moment he did not know.

From the other standpoint, I did point out that I thought that during an outbreak it would be practically impossible to learn anything if one began using a placebo when working with an active immunizing agent, which is going to require time before immunity develops This is too late You have to do it before the outbreak

This is not the same as using gamma globulin, where the protection begins, essentially, immediately after the injection, when one can use placebo injections and enter into a beginning epidemic episode and obtain results with placebo

These are quite different, and this is what I meant to point out

Dr Bell I wonder if Dr Hale would comment on this Here is a virus, Type 1 virus, put into the community which should have replaced the other virus, as Type 2 did, apparently, in his situation in Singapore; and here it certainly did not have any effect as far as I can see There is no evidence of any curtailment or interference effect at all with the homologous virus, whereas you used the heterologous virus and got some interference effect.

Chairman Gear: Would Dr. Hale like to comment now?

Dr Hale No.

Chairman Gear: Dr. Cox?

Dr. Cox: I would like to ask what dosage of CHAT strain virus was fed, were actual titrations made, and if so, what were the titers per dose? We all know how important dosage is in establishing intestinal infection.

Chairman Gear: Dr Sabin?

Dr. Sabin: I merely want to say a word about interference I think, on the basis of military operations, that one would not expect an army of 1,000 to interfere with an army of 100,000, particularly if you sent that army of 1,000 in dribbles, so that they could easily be wiped out.

Whether on purpose or otherwise, the feeding here was done slowly Am I correct?

It was started in August and the whole 75,000 were extended over a long period of time, and it is quite a different thing from doing that and doing a mass of at least 80 per cent in a short period of time.

I think this shows that one just cannot judge This is a situation which, it seems to me, does not lend itself to analysis, with one exception—*which I think we will also find later on, and also* on the basis of data previously presented—that obviously there was interference with the effect of the vaccine

It is known that when this same vaccine is given to children under conditions where there is no interference, close to 100 per cent can develop antibodies It is quite obvious here that this did not occur, and it is also obvious—the one thing about which there would be no disagreement—that some of the children who had the vaccine before, three months later developed paralysis

I think this is precisely the sort of condition that one would expect where other agents in the community would interfere with the vaccine strains when they are administered solely, and when there is an infection, the non-immunized ones would be expected to be non-immune

If a decision would have to be made on such a trial, the answer would have to be no If this is all the evidence there was, one would obviously say no

Dr Lebrun *(through an interpreter)* I would like to stress one fact in particular The 1958 59 epidemic stopped for the first time, became localized within the city I have followed epidemics since 1950, and this is the first time that I saw this phenomena, the first time I saw polio, become localized in one section of the city

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I think Dr. Plotkin has pointed out some of the difficulties in interpreting the problem There is at least one additional factor Although he calculated "weeks of exposure" for the vaccinated and non-vaccinated, these "person-week exposures" were not always at the same level of risk as time progressed

The risk of each of those persons was changing, as one drew near the end of the epidemic and the number of vaccinated was greatest, and therefore the largest number of vaccinated persons was accumulating "weeks of exposure," the risk of exposure was rapidly decreasing. The risk was greatest earlier when there were fewer "weeks of exposure" of vaccinated individuals

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FOURTH SESSION

THURSDAY, 25 JUNE 1959

Chairman
DR. ANDREW J. RHODES
Director, School of Hygiene
University of Toronto
Toronto, Ontario, Canada

TOPIC V. FIELD TRIALS *(continuation)*

Presentation of Papers by:

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Dr Miguel López Berrios
(DISCUSSION)

Dr Manuel Ramos Alvarez
(DISCUSSION)

Prof F Przesmycki
(DISCUSSION)

Dr José Manuel Quirce
(DISCUSSION)

Dr. M. K Voroshilova
Dr. Vilém Skovránek
(DISCUSSION)

Dr. Herald R Cox
for
Dr. N. Oker-Blom
(DISCUSSION)

However, this section is the very section where we did not vaccinate. Therefore, it is difficult to believe that the vaccination did not act as a protection.

On the other hand, if you mean that the slowness of the feeding is responsible for the epidemic by permitting an increase in the virulence of the strain by passages, you should explain why this strain increased and decreased in virulence so quickly and only in this section of the city where the virus should be brought by carriers, and not in the section where the strain was deliberately introduced.

Insofar as interference is concerned, it is true that there is interference, not only with the known strains of polio, but also possibly with other viruses circulating in the community

As Dr Boyd said yesterday, when we are in charge of public health, we do not act as laboratory scientists, but in view of what happened, I repeat that now, after my nine years of experience in Leopoldville, this is the first time I saw that phenomena taking place, just this year I can say only one thing that I am absolutely personally convinced that the strain of the living vaccine which was given in Leopoldville is efficient and without danger

Dr Plotkin First, in answer to Dr Hammon's point concerning placebo personally I could not agree with you more, but as you know it is sometimes difficult, for one reason or another, to use a placebo experimental design Your point about the change in risk with the increase in the numbers vaccinated is also absolutely well taken, it is a factor which one cannot easily take into account I presented this material because I wished to have it discussed I hoped, in other words, to evoke some suggestions about how to deal with this problem.

However, I would remind the previous discussants that there were three populations involved in Leopoldville, and in one of them vaccination was in full force before the epidemic As I stated earlier, one could exclude the parts of the city where the epidemic was close on the heels of vaccination, and still there appears to have been some protective effect.

In answer to Dr. Anderson, I have no idea of what the degree of spread was. It does not appear to have been very considerable If it had been, I think the percentage of sero-conversion in the vaccinated children would have been greater, because these children were in contact with vaccinated siblings

With regard to Dr Cox's question, the dosage employed was 5 logs of virus

The relation of the epidemic to the vaccination campaign is obviously worthy of considerable careful thought The point which I attempt to make was simply that one the basis of the available epidemiological and laboratory evidence, leaving aside possible conjectures, there does not appear to have been any relationship between the vaccination and the epidemic. The epidemic was, as far as I can judge, entirely consistent with what has occurred before in Leopoldville

I did not mention that the peak of this epidemic may have been higher than previous ones because of better reporting Certainly the physicians of Leopoldville were highly sensitized to polio during the vaccination campaign At any rate, the current epidemic was similar to previous ones, though perhaps slightly higher in magnitude, but then, sanitary conditions in Leopoldville are also improving.

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7. COMMUNITY-WIDE VACCINATION PROGRAM WITH ATTENUATED POLIOVIRUS IN ANDES, COLOMBIA*

HÉCTOR ABAD GÓMEZ, M.D., FRANCISCO PIEDRAHITA, M.D., RODRIGO SOLÓRZANO, M.D. AND MAURICIO MARTINS DA SILVA, M.D.†

DR ABAD GÓMEZ *(presenting the paper)*. Mr. Chairman, ladies and gentlemen, before presenting our paper on two experiments and trials, I should like to tell you what we did, how and why, under two situations, one of which called for immediate and urgent action

As Health Officer of the State of Antioquia, Colombia, I faced an outbreak of polio in the town of Andes, and found myself with a dilemma to solve: either to do something to try to detain an "impending epidemic," or to let a wild, bad, unexpected virus go ahead killing and paralyzing many children

I knew that the Salk vaccine was good, and I asked for it. But, if I tried to protect the population of our state with such a measure, we would have had to expend the total budget of the Health Department, or even more, in that action

I was then offered by the Pan American Sanitary Bureau a better vaccine, which had neurovirulence for monkeys, to be sure, although not as much had been reported as we have learned about here It was, however, an infinitely better vaccine—so I was assured—as shown in a group of Minnesota students and their children

The visit of Dr Fred L Soper, Director of the Pan American Sanitary Bureau at that time—who gave us courage while, paradoxically, warning us that the vaccine did have intraspinal neurovirulence for monkeys—a cable from Dr. Gaylord Anderson, stating that he believed the virus was safe, and the enthusiasm and vigor of Dr Mauricio Martins da Silva, were the decisive factors in our final decision to go ahead

I assure you, however, that this was not an easy decision to make, and that every telephone call to my office or home from the town of Andes during the first three months of the vaccination program, was tensely received

I am grateful that we had a vaccination team leader in the field, Dr Francisco Piedrahita, whose cheerful voice at the very beginning of every conversation, and the data he constantly gave us, reassured me that things were going absolutely all right

They continued to be so and have been so thus far, and this gave us the basis to start a larger vaccination campaign in the city of Medellín, the preliminary results of which I am also going to report today

In connection with this last report, I might even give to Dr Paul a case or two, which I believe he was asking for yesterday, to ponder over the possibility that the cases might be related to the vaccine itself

And, finally, I would like to confess, before starting the reading of these reports, that at times during this Conference, especially while hearing Dr Dick, Dr Bodian, Drs Melnick, and Dr Langmuir, I have asked myself, "My Lord, what have I done?"

The text of the first paper is as follows

In Colombia paralytic poliomyelitis has been regarded generally as a sporadic disease of minor

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bidity from typhoid fever was 425 per 100,000 in 1957 (Antioquia Health Department Reports).

Material and Methods

A project headquarters was set up in the town of Andes with a full time staff of 3 physicians (including 2 epidemiologists the project director and the PASB project consultant), one graduate nurse and 6 nurse aids Other personnel included a laboratory technician, a health educator, an observer-virologist from the laboratory that produced the vaccine, and the PASB regional adviser on poliomyelitis Cooperation of community leaders, particularly those of the church, was enlisted through careful explanation of the objectives of the program and the details of the plan of operation.

Vaccination was started on 5 May 1958 In the town of Andes, vaccination was carried out on a house-to-house basis. Elsewhere, the inhabitants of each area gathered at one of the 19 vaccination points, usually a school or church Five of these vaccination centers were beyond the limits of the county of Andes, in areas where cases of paralytic poliomyelitis had developed.

Vaccination was limited to children between the ages of 2 months up to and including 6 years Children under 7 years of age had been shown to be, serologically, the group most susceptible to the disease[3,4] Furthermore, all cases of paralytic poliomyelitis in the present Andes outbreak occurred in children under 6 years of age In view of the high infant mortality rate in the area, particularly in the first 2 months of life, infants under 2 months of age were excluded to avoid association of vaccination with illness or death due to unrelated causes The consensus was that these babies would be subjected to no unusual risk, since they would be protected by maternal poliovirus antibodies[15-17]

For field trial purposes, the county was divided into 16 sections Before vaccination, a census was taken of all families that agreed to have their children vaccinated. Blood samples were taken from the children to be vaccinated and from their siblings aged 7 to 9 years, inclusive The latter group served as contacts in whom evidence of virus spread might be sought in the post-vaccination period

A record was kept of the following information for each child vaccinated and each contact bled name of each subject, age, sex, parents' names, address, dates of vaccination and bleeding, and post-vaccination clinical observations The epidemiological follow-up and bleeding of vaccinated children and their contacts was conducted in collaboration with the Health Center of Andes and with the assistance of the 7 physicians who practice in the area

At the time of first vaccination, parents were instructed that in the event of any illness in the family after the administration of the vaccine they should apply for medical advice and assistance either to the Health Center, or to a temporary center especially established for this project. As a result, attendance at the health centers increased greatly and medical surveillance in the area was generally improved After the feeding of the Type 1 vaccine, participants in the program were observed and questioned at intervals of 3 or 4 weeks, that is, at the times of feeding the Type 2 and Type 3 vaccines and on collection of the post-vaccination blood samples In the town of Andes, members of the nursing or medical staff of the project made visits every 2 weeks to the homes of participants from the time of the first feeding of vaccine through the collection of post vaccination blood samples and entered any pertinent medical data on the individual records Since the completion of the vaccination program, observation of all vaccinated and contact children has been continued through the cooperation of the staff of the Health Center, the locally practicing physicians, and the alerted community leaders

The attenuated strains of poliovirus Type 1 (SM strain), Type 2 (MEF₁ strain) and Type 3 (Fox strain) were used in the vaccine The passage histories of these strains were identical to those used in the 1958 Minnesota trial[18] The vaccine was dispensed in hard gelatin capsules in which the virus was adsorbed on granular gelatin

The vaccination program was carried out in three steps Children were fed first one capsule containing $10^{6.8}$ $TCID_{50}$ (50 per cent tissue-culture-infective dose) of Type 1 virus (Pool No 7-1231-115A), the virus type isolated during the Andes outbreak, 4 weeks later they were given two capsules containing a total of $10^{5.8}$ $TCID_{50}$ of Type 2 virus (Pool No 7-1232-213), and 3 weeks thereafter they received one capsule containing $10^{5.1}$ $TCID_{50}$ of Type 3 virus (Pool No

public health importance. The number of cases reported annually from 1947 to 1957[1, 2] ranged from 29 to 169, the highest rate being 1 6 cases per 100,000 population, in 1953.

In contrast to the fact that the paralytic form of the disease is rarely seen, poliovirus infection was shown to be almost universal in parts of the country by the age of two years.[4, 5] Surveys of the distribution of naturally occurring poliovirus antibodies in Cali and Bogotá indicated that all children two years of age or older had antibodies to one or more types of poliovirus, and that in the 7 to 9 year-old group, 88 per cent already had antibodies to all three known types Comparable results have been reported in studies of other areas of the world with similar socioeconomic and hygienic conditions[6-9]

Early in January 1958, the Health Center of Andes, a municipio (county) in southern Antioquia, Colombia, with a population of 50,000, began to report cases of paralytic poliomyelitis By March, 9 cases and 3 deaths had been recorded Alerted by these reports to the possibility of an incipient epidemic, the health authorities of the Departamento (State) of Antioquia and the Ministry of Public Health of Colombia requested the Pan American Sanitary Bureau (PASB) to cooperate in a vaccination program making use of the inactivated poliovirus vaccine

In conformity with the request, one of the authors (M M S) visited Andes on March 19 to evaluate the situation and assist in identifying the type of poliovirus involved in the outbreak Stool and blood samples were collected from six children with paralytic disease and all members of their households Within a week of collection of the samples, Type 1 poliovirus had been isolated from the stools of 2 children with the disease and 4 contacts, and this information was sent to the health authorities in Colombia

Because the formalinized vaccine is incapable of halting the spread of poliovirus in a community,[10, 11] and therefore could not be expected to alter the course of an epidemic, and since it seemed likely that an outbreak was in progress, a plan was developed to vaccinate children of the more highly susceptible age groups in Andes with orally administered live attenuated poliovirus vaccine The plan was formulated on the basis of previous experience with the vaccine in Minnesota[12, 13] and of suggestions of the Expert Committee on Poliomyelitis of the World Health Organization[11] regarding the field uses of live poliovirus vaccines "in the face of an impending epidemic" Additional reasons for advocating this type of vaccine were: a) the relative simplicity of oral administration of vaccine; b) the long lasting protection apparently conferred by the vaccine, as measured by circulating antibodies,[14] making less frequent, or perhaps even unnecessary, the need for booster doses; and c) its capacity to induce within the alimentary tract a high degree of resistance to the virus, thus offering the possibility of reducing and eventually eliminating the spread of virulent paralytic strains of poliovirus from the community.

Geographical and Population Data

The Municipio of Andes is situated at the southwestern tip of Antioquia and covers an area of approximately 241 square miles of extremely mountainous terrain with altitudes ranging from 3,300 to 11,500 feet The principal community, the town of Andes, is connected by an unsurfaced road with Medellín, the capital of the state, 83 miles to the north

Andes is a typical rural, agricultural county, with coffee as the principal cash crop. As in many South American coffee-growing areas, the production of essential foodstuffs is negligible, and dietary deficiencies and malnutrition are widespread.

The county contains 20 population groupings, 8 of which can be reached by automobile and 12 only by mule trail Of the estimated population of 50,000, approximately 7,000 live in the town of Andes and the remainder in rural areas. In the urban area, 54 per cent of the dwellings have bathing facilities, 74 per cent have latrines or privies and 91 per cent have water available on the premises. In rural areas, housing is universally poor, lacking running water and sanitary privies

The median family consists of 7 members In the study area the distribution of the population by age group is 37 per cent under 7 years, 11 per cent between 7 and 10 years, and 52 per cent over 10 years, as determined by the census made as a part of this study. The reported mor-

7 1232-318) For the feeding of small children, the capsules were opened and the contents were mixed with water and administered by spoon

In order to ascertain the immune status of the population, prior to the administration of the Type 1 poliovirus vaccine, blood samples were collected from 690 individuals, all but 61 of whom were between 6 months and 10 years of age These specimens were obtained throughout the study area by bleeding the children within this age group in every tenth family among the 2,922 families participating The data regarding these sera are assembled in Table 1

Five hundred ninety-one of the 690 individuals who were bled initially were re bled one month after the administration of the third dose, i.e., Type 3 poliovirus vaccine The pre- and post vaccination blood specimens were titrated in 4-fold dilutions to determine the immunologic response of the children to the vaccine and the extent of spread of the vaccine strains to the household contacts

The paired samples were tested as units of two by the method of Salk, Youngner and Ward[18] with use of monkey kidney-cell cultures Each 4-fold dilution of serum was tested in duplicate tubes against 100 to 300 $TCID_{50}$ of one or another of the three types of poliovirus Fifty per cent neutralizing endpoint were calculated by the method of Reed and Muench[19] and the results reported to the nearest two-fold dilution Serologic data thus obtained are summarized in Tables 2, 3 and 4 An antibody titer of 1:4 or greater in the post-vaccination specimens of children previously lacking demonstrable antibody was considered a positive response In children with pre-vaccination antibodies, a 4-fold or greater rise in titer was considered a booster response

Observations and Results

Response to Vaccination—The numbers of children vaccinated were 7,378 with Type 1 virus 7,122 with Types 1 and 2, and 6,977 with all three types Only 5.5 per cent of the children who started the vaccination schedule did not complete it

The distribution of naturally occurring poliovirus antibodies in 690 Andes residents prior to vaccination is presented in Table 1 Antibodies were not detected for any type of poliovirus in the sera of 9.7 per cent of the children Antibodies were present for one or two types of virus in the sera of 35.5 per cent, and for all three types in 54.8 per cent. Thus 45.2 per cent of the children lacked demonstrable antibody for one or more types of poliovirus

The poliovirus antibody status of vaccinated children, before and after feeding of vaccine, is summarized in Table 2 and Figs 1, 2, and 3 It can be seen that a post-vaccination conversion from negative to positive occurred in 91 per cent for Type 1, 72 per cent for Type 2, and 87 per cent for Type 3 virus Booster responses were as follows in children with pre-vaccination titers ranging from 1:4 to 1:16 75 per cent for Type 1, 61 per cent for Type 2, and 81 per cent for Type 3, in children with pre vaccination titers ranging from 1:32 to 1:512, 43 per cent for Type 1, 57 per cent for Type 2, and 52 per cent for Type 3

The antibody status of household contacts, before and after the vaccination of the index children, is summarized in Table 3, and Figs 1 and 2 These data show a conversion from negative to positive in 6 of 9 for Type 1, 2 of 3 for Type 2, and 1 of 6 for Type 3 Relatively lower conversion rates for Type 3 might be expected, because the second bleedings took place only one month after the feeding of Type 3 vaccine and hence there was less time for contact exposure and for serologic response to that virus strain. In the contacts who showed evidence of natural immunity at the beginning of the vaccine feedings, second samples of serum showed a 4-fold or greater booster response as follows in those with antibody titers originally ranging from 1:4 to 1:16, 67 per cent for Type 1, 60 per cent for Type 2, and 36 per cent for Type 3, in those with an initial range from 1:32 to 1:512, 25 per cent for Type 1, 30 per cent for Type 2, and 35 per cent for Type 3

The data on pre- and post-vaccination antibody status of vaccinated and contact children are presented graphically in Figures 1 and 2. The serologic data for vaccinated children are summarized by age in Table 4, and part of these data are presented graphically in Figures 3 and 4

The usual decline in antibody for Types 2 and 3 poliovirus among children in the 6-to-11-month

TABLE 1 PERCENTAGE DISTRIBUTION OF NATURALLY OCCURRING POLIOVIRUS ANTIBODIES BY IMMUNOTYPE AMONG CHILDREN BY AGE GROUP ANDES, COLOMBIA, 1958

Age Group		Number of Sera or of Children	Percentage of Sera With Antibodies to Poliovirus Type							Percentage Without Antibody to Types 1, 2 or 3
			Three Types	Two Types			One Type Only			
			Types 1, 2 and 3	Types 1 & 2	Types 1 & 3	Types 2 & 3	Type 1	Type 2	Type 3	
6–11 months		40	5.0	10.0	7.5	7.5	22.5	10.0	2.5	35.0
1 year		43	0	11.6	2.3	0	32.6	0	4.7	48.8
2 years		68	26.5	7.4	5.9	8.8	14.7	11.8	2.9	22.0
3 years		82	36.6	13.4	9.8	7.3	13.4	7.3	4.9	7.3
4 years		91	52.7	14.3	6.6	9.9	4.4	3.3	4.4	4.4
5–6 years		180	66.7	12.2	2.2	6.7	5.6	0.5	3.3	2.8
7–9 years		125	84.0	5.6	1.6	6.4	0.8	0	0	1.6
10 years and over*		61	90.2	6.6	1.6	0	0	1.6	0	0
Total	Number	690	378	71	29	44	59	23	19	67
	Per Cent	100	54.8	10.3	4.2	6.4	8.5	3.3	2.8	9.7

* Includes a small number of adults

TABLE 3 ANTIBODY RESPONSE OF UNVACCINATED CONTACTS BY IMMUNOTYPE
ANDES, COLOMBIA, 1958

PRE-FEEDING TITERS	TYPE 1			TYPE 2			TYPE 3		
	TOTAL NUMBER	POSITIVE RESPONSES 4x OR >		TOTAL NUMBER	POSITIVE RESPONSES 4x OR >		TOTAL NUMBER	POSITIVE RESPONSES 4x OR >	
		NUMBER	PER CENT		NUMBER	PER CENT		NUMBER	PER CENT
<1 4	9	6	67	3	2	67	6	1	17
1 4	1	1	100	5	3	60	2	1	50
1 8	4	3	75	3	1	33	15	5	33
1 16	7	4	57	7	5	71	11	4	36
1 32	25	9	36	20	8	40	42	19	45
1 64	10	3	30	10	2	20	6	1	17
1 128	37	10	27	31	12	39	32	11	34
1 256	10	2	20	9	1	11	5	1	20
1 512	21	2	10	20	4	20	11	2	18
1 1024	a) 2	—	—	b) 7	—	—	c) 1	—	—
>1 1024	a) 8	—	—	b) 19	—	—	c) 3	—	—
Total	a) 124	40	32	b) 108	38	35	c) 130	45	35

(a) 10 sera are omitted from total (b) 26 sera are omitted from total (c) 4 sera are omitted from total

TABLE 2. ANTIBODY RESPONSE OF VACCINATED CHILDREN BY IMMUNOTYPE
ANDES, COLOMBIA, 1958

Pre-Feeding Titers	TYPE 1			TYPE 2			TYPE 3		
	Total Number Fed	Positive Responses 4x or >		Total Number Fed	Positive Responses 4x or >		Total Number Fed	Positive Responses 4x or >	
		Number	Per Cent		Number	Per Cent		Number	Per Cent
<1:4	128	116	91	151	108	72	179	156	87
1:4	8	6	75	2	1	50	10	8	80
1:8	16	13	81	29	20	69	47	38	81
1:16	12	8	67	5	1	20	13	11	85
1:32	48	34	71	41	28	68	70	44	63
1:64	16	5	31	8	6	75	17	15	88
1:128	79	44	56	55	34	62	64	32	50
1:256	36	9	25	15	8	53	12	4	33
1:512	68	14	21	56	23	41	32	7	22
1:1024	a) 11	—	—	b) 12	—	—	c) 2	—	—
>1:1024	a) 30	—	—	b) 81	—	—	c) 11	—	—
Total	a) 411	249	61	b) 362	229	63	c) 444	315	71

(a) 41 sera are omitted from total (b) 93 sera are omitted from total (c) 13 sera are omitted from total

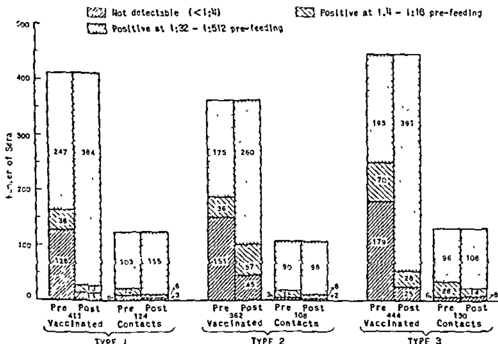


FIG. 1 Poliovirus antibody response in vaccinated and contact children, before and after feeding of vaccine, Andes, Colombia, 1958

age group was not paralleled by the curve of the Type 1 antibody This may reflect the fact that Type 1 virus had been prevalent in the community for several months before the collection of the prevaccination specimens That this higher percentage of children having Type 1 antibody is associated with recent infection may be seen in Table 4, where the proportion of pre-vaccination titers of more than 1 64 is far greater for Type 1 than for Types 2 and 3 among the children under 2 years of age

A study of individual serologic records, revealed that, among the 128 children without demonstrable antibody for Type 1 virus before feeding, 6 failed to respond to both Type 1 and Type 2 vaccine strains and 2 others failed to respond to both Type 2 and Type 3 vaccines Of the 151 children lacking Type 2 antibody before vaccination, 9 failed to respond to both Type 2 and Type 3 vaccine strains Twelve of these children who failed to respond to 2 of the 3 vaccine strains lacked antibody for any of the three serologic types of poliovirus prior to vaccination Failures to respond were most frequent for the Type 2 vaccine strain In addition to those cited above, 27 children all but five of whom were under 4 years of age, failed to respond to Type 2 only despite the absence of antibody for this virus type in their prevaccination sera Sixteen of these 27 children also lacked Type 1 antibody at the start of the feeding program

A survey of the individual vaccination records of those children who failed to respond to vaccination did not reveal significant grouping of failures in time, place or vaccine consignment They were randomly distributed over the study area and hence are not suggestive of lack of response resulting from defective or mishandled vaccine or of the localized prevalence of interfering enteric infections, which, presumably, could be found to predominate in the urban segments of the town of Andes

Epidemiologic Observations —At the start of the

Table 4. Per Cent Distribution and Mean Titer of Sera by Immunotype and Age Before and After Live-Attenuated Poliovirus Vaccination in 428 Children—Andes, Colombia, 1958

Age (and Number) of Children	Number of Sera	Type of Virus	Per Cent of Sera: Titers <1:4		Titers 1:4 to 1:64		Titers >1:64		Geometric Mean Titer	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
<1 year (36)	36	1	61	6	14	11	25	83	6	267
	36	2	67	36	33	45	0	19	2	8
	36	3	75	17	22	53	3	30	2	29
1 year (40)	40	1	55	5	0	80	45	87	15	305
	40	2	93	22	7	53	0	25	1	16
	40	3	93	15	5	33	2	52	1	45
2 years (60)	59	1	44	10	19	20	37	70	15	131
	60	2	42	13	15	27	43	60	25	129
	60	3	57	5	28	52	15	43	5	54
3 years (71)	70	1	31	1	14	9	55	90	38	291
	71	2	37	8	15	24	48	68	34	195
	70	3	41	1	36	30	23	69	11	186
4 years (75)	74	1	23	1	31	20	46	79	42	104
	74	2	19	3	19	20	62	77	95	260
	75	3	23	3	40	23	37	74	28	201
5 years (77)	77	1	16	1	31	22	53	77	56	192
	75	2	19	7	17	14	64	79	112	294
	76	3	26	3	39	27	35	70	22	149
6 years (69)	68	1	9	2	23	22	68	76	295	200
	69	2	10	1	23	16	67	83	136	338
	69	3	18	4	43	33	39	63	32	173

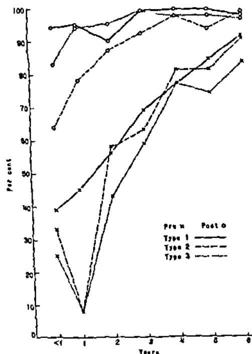


Fig 3. Percentage distribution of poliomyelitis antibodies by immunotype, before and after vaccination, in 428 children, by year of life, Andes, Colombia, 1958

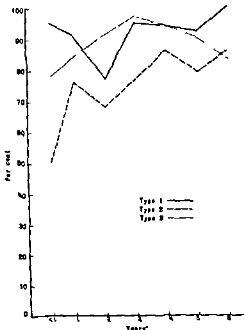


Fig. 4 Children with type specific poliomyelitis antibodies after vaccination as a percentage of those without demonstrable antibodies before vaccination, by year of life, Andes, Colombia, 1958

cination program had been extensively tested in various ways in monkeys before their administration to human subjects While the relationship between virulence for monkeys and pathogenicity for man is difficult to assess, studies of a field strain of Type 1 virus that was isolated in Andes prior to the vaccination program from one of the children with paralysis are interesting As little as two $TCID_{50}$ of this field strain produced complete paralysis when injected intracerebrally in monkeys, whereas 10 million $TCID_{50}$ or more of any of the three attenuated strains used as vaccine rarely induce even slight paralysis when similarly injected [20]

Laboratory studies in experimental animals are important and essential, but only field experience on a scale such as that gained in Andes provides an acceptable basis for determining the advisability of the continued use of the viruses employed The safety of the vaccine strains for human subjects had been ascertained before their use in Andes by small-scale trials in children and adults, totalling about 1,000 persons, of which the most recent are those in Minnesota,[14] where the behavior of the strains was observed under close supervision in the households of approximately 150 married university students and their children, and in another earlier trial involving 25 infants and their family contacts [11]

The results attained in these studies were the justification for making a larger trial The data obtained in the present field study confirm the observations made in the former trials and greatly add to the number of susceptible children safely vaccinated

The pre-vaccination serologic data summarized in Table 1 show that, of the 504 children under 7 years of age, 65 or 12 8 per cent lacked detectable antibody to any of the three types of poliovirus. Most of these fully susceptible children were under 4 years of age By applying the same percentage of susceptibility to the 7,378 children who received Type 1 virus, it can be estimated

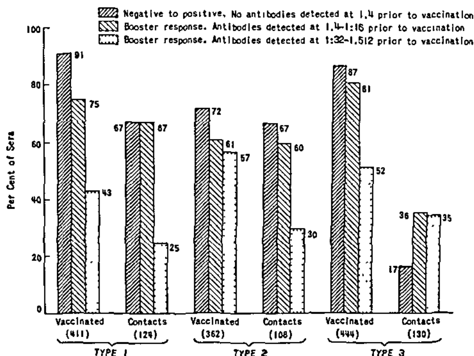


FIG 2 Percentage of sera in vaccinated and contact children with a minimum four-fold antibody titer increase after the administration of live attenuated poliovirus vaccine, Andes, Colombia, 1958

vaccination program, 15 verified cases of poliomyelitis had been reported from the area After 5 May, when the program began, four additional cases were reported from sections where the oral vaccine had not yet been administered.* Reported cases occurred in children ranging in age from 5 months to 6 years, with a median age of 1 year Up to the time of writing (17 June 1959), no cases of poliomyelitis had been recorded among vaccinated children or their contacts

Vaccinated children showed no untoward reactions. The post-vaccination gastro-intestinal complaints (such as diarrhea and vomiting) occasionally reported at the health centers or during follow-up visits were no more frequent than would normally be expected in a population highly infested with helminths and intestinal protozoa In the few instances where symptoms were sufficiently severe to warrant further investigation, stool studies performed at the State Health Laboratory in Medellín frequently revealed parasitic infestation or the presence of pathogenic bacteria

During the vaccination program (May to August 1958), 17 deaths were recorded among children who had received at least one dose of the vaccine. 9 children were less than one year of age, and the age of the others ranged from 1 to 5 years Diagnosis listed on death certificates by attending physicians were gastroenteritis in 9 cases, pneumonia and bronchitis in 2 cases, and glomerulonephritis, diphtheria, and an overdose of Nivaquine (an antimalarial drug) in one case each The other three children were unattended by a physician, but query of the parents revealed that none showed any history suggestive of poliomyelitis or other central nervous system involvement. Seventeen deaths during a period of 4 months, in a population of approximately 7,000 children is less than expected in Andes, where in 1957 the infant mortality rate was 121 per 1,000 live births and the mortality in the 1-to-4-year age group was approximately 20 per 1,000 population

Discussion

The strains of viruses used in the Andes vac-

* See in map attached, cases No. 16, 17 and 19 in Jardín, and No 18 in Hispania

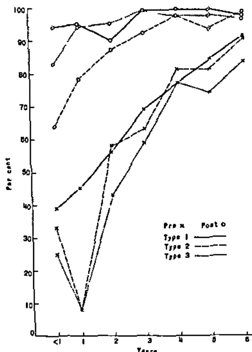


FIG 3 Percentage distribution of poliomyelitis antibodies by immunotype, before and after vaccination, in 428 children, by year of life, Andes, Colombia, 1958

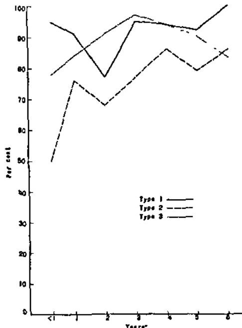


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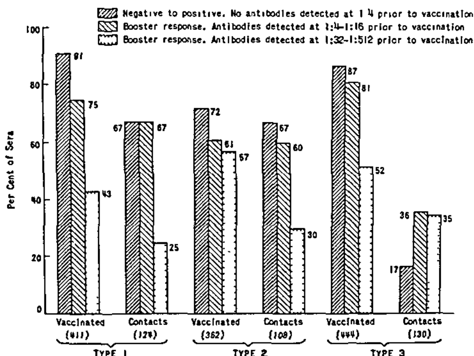


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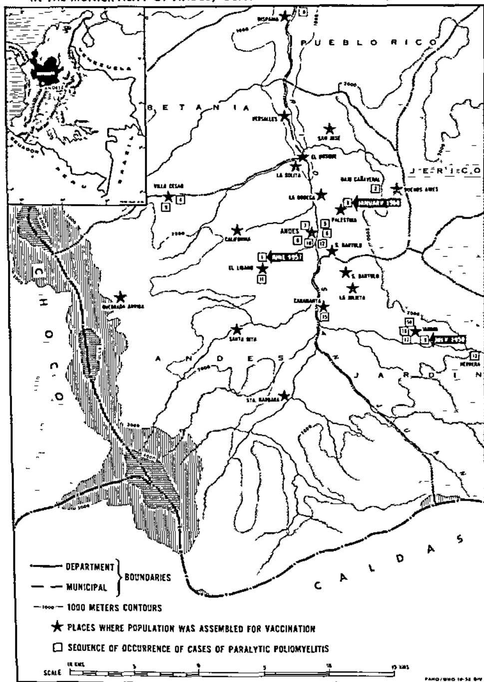
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Discussion

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VACCINATION WITH LIVE ATTENUATED POLIOMYELITIS VACCINE
IN THE MUNICIPALITY OF ANDES, DEPARTMENT OF ANTIOQUIA, COLOMBIA.



that 952 triple negative children were vaccinated with this strain. Similarly, it is estimated that an additional 34 triple negative children were among the 2,144 siblings aged 7 to 10 years who were in intimate contact with the vaccinated children, most of whom, it may be assumed, excreted Type 1 virus for varying periods of time Thus an estimated 986 triple negative children in the study area are expected to have been exposed to the Type 1 strain of the vaccine

On the basis of the data summarized in Table 2, it may be calculated that 28 3 per cent, or 2,088 children, fed Type 1 virus were without homologous antibody protection at the time of feeding In a like manner, an estimated 33 1 per cent, or 2,357 children, were without antibody to Type 2, and 39 1 per cent, or 2,728, were without antibody to Type 3 when fed these strains The high rates of conversion (Fig 4) indicate that vaccination successfully infected a very large proportion of these susceptible children Among 58 children who were originally triply negative and were re-bled after vaccination, 91 per cent responded to Type 1, 64 per cent to Type 2, and 86 per cent to Type 3 poliovirus vaccines There remained no triple negative children among those tested after vaccination

In the absence of any observed indications or signs of central nervous system or other complications in the vaccinated children and their contacts, we believe that continued use of the polioviruses employed in Andes is amply justified

The circumstances under which the present vaccination program was initiated justified an immediate and direct defense against a Type 1 poliovirus epidemic. While it cannot be proved that in the absence of the vaccination program an epidemic would have developed and no claim is made to have aborted such an epidemic, the measures taken were selected for that purpose For this reason the Type 1 vaccine was fed first, in spite of the fact generally held that the attenuated SM strain of Type 1 virus may be sufficiently invasive to "interfere" with subsequent infection by virus of the other two immunotypes

In the present study, the proportionately greater number of children failing to respond to Type 2 strain suggests that Type 1 strain interfered with the successful establishment of Type 2 in the intestines of those lacking antibody to this virus. Also, it may be seen in Table 4 that for children under 2 years of age the increment in geometric mean titer for Type 1 is much greater than for the other two types. Since it is clear that the relatively lower titers for Types 2 and 3 are not due to any inability of children of this age to respond to antigenic stimulation, the recency of their experience with the Type 1 vaccine virus may have been a contributing factor in determining their lesser response to the other vaccine strains fed

In the absence of epidemic threat it appears to be preferable to start an immunizing program with Type 2 virus, both because it may have some antigenic overlapping with the other two types of poliovirus,[21, 22] and because the Type 2 strain used in this study seems to possess a lesser degree of infectivity than either the Type 1 or Type 3 strains The indicated order of feeding would therefore appear to be either Types 2, 3, 1, or 2, 1, 3 The data in Table 4 for children over 3 years of age show that, in spite of the antigenic overlapping of Type 2, antibody for this type, even at relatively high prevaccination levels, did not prevent substantial increments in mean titers for Types 1 and 3, nor indeed for Type 2 itself

Summary and Conclusions

In the face of an outbreak of poliomyelitis in Andes, Antioquia, Colombia, an oral vaccination program was carried out on a community-wide basis with attenuated strains of poliovirus representing each of the 3 virus types With the participation of local, national, and international health organizations, a staff was assembled to take a census of the area, administer the vaccine, collect pre- and post-vaccination serum samples from vaccinated and contact children between the ages of 6 months and 9 years, and maintain epidemiologic surveillance of the affected area Of 7,378 children who began the series of vaccinations, 94 5 per cent completed the three immunizing feedings.

Serologic evidence indicates that, of the vaccinated children who lacked demonstrable type-specific antibody at the time of vaccination, 91 per cent responded to Type 1 virus, 72 per cent to Type 2, and 87 per cent to Type 3 Smaller but significant proportions of those with prior

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demonstrable antibodies showed 4-fold or greater booster responses to vaccination. Of 58 children shown to be negative before vaccination to all 3 types of poliovirus, none remained so after vaccination

Although four new cases of poliomyelitis were reported in the Andes study area after the start of the oral vaccination program, none of the cases occurred in a vaccinated child or a contact, nor did any of the cases develop in areas where vaccination had begun Since the start of the vaccination program in May, 1958, there have been no undesirable reactions associated with or *attributable to the oral vaccination.*

Both the safety and the immunizing efficacy of the strains of virus employed were demonstrated by their performance in an estimated 986 vaccinated or contact children who were without serologic evidence of previous exposure to any type of poliovirus, and in between two and three times this number of children who lacked antibody to one or more types of poliovirus

The vaccine used in this study was provided by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., through an agreement with the Pan American Sanitary Bureau

The specimens were flown under refrigeration *to Dr. Herald R Cox at the Viral and Rickettsial* Research Section of Lederle Laboratories Dr Cox and staff carried out the virus isolation and antibody titrations reported.

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8. VACCINATION OF 133,000 CHILDREN UNDER 10 YEARS OF AGE WITH LIVE ATTENUATED POLIOVIRUS IN MEDELLIN, COLOMBIA—PRELIMINARY REPORT

HÉCTOR ABAD GÓMEZ, M.D., DAGOBERTO GAVIRIA, M.D., FRANCISCO PIEDRAHITA, M.D., MARIO GALDÓS, M.D., AND MAURICIO MARTINS DA SILVA, M.D.*

DR ABAD GOMEZ *(presenting the paper)* In view of the successful outcome of the vaccination of more than 7,000 children in the Municipality of Andes, Colombia,[1] a campaign was started in September 1958 to vaccinate all children under 10 years of age in the city of Medellín, Capital of the Department of Antioquia, with an estimated population of 600,000 of which 150,000 are children between 0 and 10 years of age

Method Used—The campaign personnel consisted of 40 vaccinators, who had previously received special training in the application of the vaccine and who worked under the supervision of two public health nurses, and three full-time physicians, in addition to the consultant-epidemiologist of the Pan American Sanitary Bureau Prior to vaccination, 895 blood samples were taken from children aged 6 months to 15 years, belonging to families distributed throughout the zones into which the city had been subdivided (from 10 to 12 families in each zone) The vaccination work began on 23 September 1958 using the attenuated Type 2 (MEF₁) virus administered by mouth in liquid form in 0 5 cc doses to each child The administration of the vaccine was made on a house-to-house basis throughout the area. On 2 January 1959 vaccination with Type 3 virus (Fox strain) was started, and on 17 February 1959, the administration of Type 1 virus (SM strain) was initiated The doses administered were $10^{4.9}$ $TCID_{50}$ for Type 2, $10^{5.2}$ $TCID_{50}$ for Type 3, and $10^{4.7}$ $TCID_{50}$ for Type 1 When the program was completed on 1 April 1959, 133,642 doses of Type 2 virus, 133,557 of Type 3 virus, and 131,772 of Type 1 virus had been administered These figures represent 90 per cent of the population under 10 years of age eligible for vaccination. Four to six weeks after the administration of the three types of virus had been completed, blood samples were again collected from 730 children from whom first specimens had been obtained prior to vaccination

The distribution of poliovirus antibodies before vaccination was started is shown in Table 1

The distribution of antibodies before and after vaccination is shown in Fig 1 and Table 2

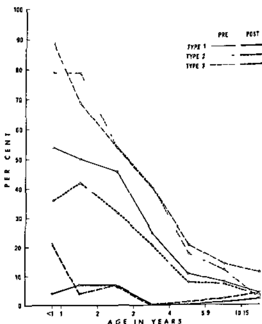


FIG 1 Per cent seronegatives in 673 sera before and after oral vaccination with live poliovirus vaccine, by age and virus type, Medellín, Colombia, 1959.

* Dr. Abad Gómez (formerly State Health Officer, Antioquia, Colombia), Dr Gaviria (State Health Department, Antioquia, Colombia), Dr Piedrahita (State Health Department, Antioquia, Colombia), Dr Galdós (Pan American Sanitary Bureau/World Health Organization), and Dr Martins da Silva (Pan American Sanitary Bureau/World Health Organization).

[1] Abad Gómez, *et al* Community-wide vaccination program with attenuated poliovirus in Andes, Colombia, *J Am M Ass 170* 906-913 (June 20) 1959

TABLE 1 DISTRIBUTION OF POLIOVIRUS ANTIBODY IN SINGLE SPECIMENS AND ALL SPECIMENS BY SEROTYPE AND AGE BEFORE LIVE POLIOVIRUS VACCINATION
MEDELLÍN, COLOMBIA, 1959

Classification of Single Specimens by Serotype	Age in Years: 6–11 Mo N	6–11 Mo %	1 N	1 %	2 N	2 %	3 N	3 %	4 N	4 %	5–9 N	5–9 %	10–15 N	10–15 %	Adult* N	Adult* %	Total N
In Single Specimens																	
None	21	51	26	39	12	13	5	6	0	0	4	1	0	0	2	2	70
1, 2, 3	2	5	7	11	13	14	31	37	51	53	281	72	121	82	89	67	595
1, 2	1	2	6	9	14	15	11	13	13	14	33	8	14	10	16	12	108
1, 3	0	0	9	14	8	9	8	10	12	13	35	9	6	4	7	5	85
2, 3	1	2	1	2	9	10	7	8	7	7	19	5	5	3	5	4	54
1	12	30	9	14	15	17	12	14	5	5	6	2	1	1	3	2	63
2	4	10	3	4	8	9	4	5	4	4	7	2	0	0	8	6	38
3	0	0	5	7	12	13	6	7	4	4	4	1	0	0	3	2	34
Total	41	100	66	100	91	100	84	100	96	100	389	100	147	100	133	100	1017
Classification of All Specimens by Serotype																	
In All Specimens																	
1	15	37	31	47	50	55	62	74	81	84	355	91	142	97	115	86	851
2	8	20	17	26	44	48	53	63	75	78	310	87	140	95	118	89	795
3	3	7	22	33	42	46	52	62	74	77	339	87	132	90	101	78	768
None	21	51	26	39	12	13	5	6	0	0	4	1	0	0	2	2	70
No of Sera	41		66		91		84		96		389		147		133		

* Cord blood

TABLE 2. DISTRIBUTION OF SERA WITH A FOUR-FOLD OR GREATER TITER INCREASE CLASSIFIED BY INITIAL TITER, SEROTYPE, AND AGE, IN 673 PAIRED SPECIMENS AFTER LIVE POLIOVIRUS VACCINATION*
MEDELLÍN, COLOMBIA, 1959

Initial Titer	Sero-type	No of Sera	Age of Child in Years																Total Responding	Per Cent Responding
			<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
<4	1	122	14	21	28	16	8	5	3	5	3	3	—	2	0	0	—	—	108	88
	2	172	12	18	15	12	7	3	2	2	2	5	1	0	—	—	0	—	79	46
	3	185	19	31	33	25	14	14	6	6	2	5	1	4	2	—	1	—	163	88
4	1	7	—	1	—	—	1	2	—	—	0	—	—	—	0	—	—	—	4	57
	2	23	2	0	0	1	2	1	0	1	1	1	—	—	—	—	—	—	9	39
	3	32	—	1	3	4	—	0	5	3	1	3	1	0	—	0	—	—	21	66
8	1	20	1	1	—	0	1	2	—	—	2	0	2	0	0	—	1	—	10	50
	2	24	—	1	0	1	1	2	1	2	2	—	—	0	0	—	—	—	10	42
	3	38	1	2	4	2	2	2	3	2	3	0	1	0	1	—	—	—	23	60
16	1	78	—	5	3	10	3	7	1	5	3	3	1	3	4	3	2	—	53	68
	2	77	—	1	3	2	7	0	4	6	4	4	1	5	3	0	1	0	41	53
	3	142	1	4	6	3	12	9	7	11	12	5	6	3	3	3	3	0	88	62
64	1	160	2	2	4	7	13	3	8	5	6	4	2	4	0	1	2	0	63	39
	2	134	—	0	5	5	10	6	6	2	6	3	4	2	1	4	0	—	54	40
	3	166	—	3	7	12	10	7	8	8	7	7	1	3	0	0	2	—	75	45
256	1	152	1	3	8	5	4	7	7	4	2	2	0	2	0	0	0	—	45	30
	2	152	—	2	6	4	9	6	8	4	5	6	0	3	1	1	2	—	57	38
	3	72	—	1	4	1	5	0	2	1	2	1	0	0	0	1	0	—	18	25
Sub Total	1	539	18	33	43	38	30	26	19	19	16	12	5	11	4	4	5	0	283	52
	2	582	14	22	29	25	36	18	21	17	20	19	6	10	5	5	3	0	250	43
	3	635	21	42	57	47	43	32	31	31	27	21	10	10	6	4	6	0	388	61
1024	1	134	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	2	91	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
	3	38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
Total	1	673	18	33	43	38	30	26	19	19	16	12	5	11	4	4	5	0	283	—
	2	673	14	22	29	25	36	18	21	17	20	19	6	10	5	5	3	0	250	—
	3	673	21	42	57	47	43	32	31	31	27	21	10	10	6	4	6	0	388	—

* Vaccination limited to children 0 to 10 years of age, children 11 to 15 years of age represent household contacts.

Epidemiological Observations—There are 570 physicians in the city of Medellín, many of whom, as general practitioners, attend children, in addition to those who are specialized in pediatrics There is a School of Medicine with 150 professors and 400 students; there are 13 health centers, one in each of the 13 areas into which the city is divided; one General Hospital with 1,000 beds that provides hospitalization for children, and various public, semipublic (Social Security Institute), and private hospitals for hospitalizing children, in addition to an intelligent and alert public opinion with respect to anything that might happen to their children During and after the vaccination program, none of the general practitioners, pediatricians, hospitals, health centers, or the School of Medicine reported any change, increase, or modification whatever in the number or type of diseases they have always treated On the other hand, with respect to poliomyelitis, the number of cases reported to the City Health Department and hospitalized have decreased, as shown in Table 3

TABLE 3 CASES OF PARALYTIC POLIOMYELITIS REPORTED IN MEDELLÍN, COLOMBIA, 1954-1959*

YEARS	NO OF CASES
1954	18
1955	12
1956	52
1957	18
1958	19
1959*	4*

* Up to 17 June 1959

With respect to deaths from all causes in the age group 0-10 years during the vaccination period, compared with the same previous five-year period, the figures are as follows

TABLE 4 NUMBER OF DEATHS FROM ALL CAUSES AMONG CHILDREN LESS THAN 10 YEARS OF AGE, FROM SEPTEMBER TO APRIL, INCLUSIVE, 1953-1959 MEDELLÍN, COLOMBIA

PERIOD SEPTEMBER—APRIL (8 MONTHS)*	NO OF DEATHS
September 1953—April 1954	1,839
September 1954—April 1955	1,970
September 1955—April 1956	2,115
September 1956—April 1957	2,334
September 1957—April 1958	2,692
September 1958—April 1959	1,518

* Covering the entire duration of the vaccination program, plus one month thereafter

Average number of deaths, September—April, inclusive (5 previous years)	2,190
The same period September 1958—April 1959	1,518

Poliomyelitis Cases During and After the Vaccination

From 23 September 1958 to 17 June 1959, 5 cases of paralytic poliomyelitis were reported in the city

A careful analysis of these cases made by the campaign personnel, revealed the following facts. *Case No 1*—B E G Female child 2 years and 4 months old—vaccinated with Type 2 virus (MEF) on 3 October 1958 Went on vacation to a city some 170 kilometers from Medellín and became ill on 23 December, 2 months and 19 days after administration of the attenuated Type 2 virus Developed paralysis of the right arm and left leg, which had almost completely recovered at last examination on 16 April of this year Stool studies during illness did not reveal the presence of any viral agent. Serum Antibodies 1 128 for Type 2, and less than 1 4 for Types 1 and 3

Case No 2—G L V Male child, eighteen months old, vaccinated with Type 2 Poliovirus on 12 November 1958 and with Type 3 on 15 January 1959 Eight days after administration of the attenuated Type 3 virus developed fever, dysphagia, and diarrhea and 18 days thereafter, complete paralysis of the right arm When seen

on 16 April, had recovered part of the movements of the fingers and wrist.

Poliovirus Type 3 and Coxsackie A4 were isolated from the stools The study of serum antibodies in two samples collected on 2 February and 4 March was negative for Types 1 and 2, and positive 1·32 for Type 3

Case No 3—L C A Seven-month-old baby girl, vaccinated with Poliovirus Types 2 and 3. Thirty-three days after receiving the Type 3 virus, developed fever, constipation, and lack of appetite and 38 days thereafter, paraplegia of the lower limbs and monoplegia of the left arm When examined on 4 April 1959, showed no residual weakness or paralysis

Examination of serum antibodies was negative for Types 1 and 2, and positive *(1 256)* for Type 3 Stool specimen was not available

Case No 4—M G D D Baby boy seven and one-half months old Vaccinated with attenuated Type 2 virus on 26 November 1958 and with Type 3, on 29 January 1959 On 15 March 1959, two and a half months after the last vaccine administration, developed high fever and constipation for three days and on 17 March, flaccid paralysis of the right leg

When examined on 16 April 1959 had recovered movement of the right foot only

Type I virus was isolated in feces on 21 March 1959 Antibodies in the serum, on 21 March showed Type 1, 1 128, Type 3, 1·512, Type 2, 1·32

Case No 5—A M E,Ch Female child, two and a half years old, vaccinated with Type 2 Poliovirus on 2 September 1958, with Type 3 in January 1959, and with Type 1, on 16 March 1959. On 9 May of this year, two months after administration of Type 1, developed high fever, diarrhea, cough and expectoration, 4 days thereafter, flaccid paralysis of the lower extremities As yet we have no laboratory results of this case

Dr. Roca-García later on will present detailed laboratory results of these cases to the Conference.

From May up to the present time (17 June 1959) we have not had a single case of poliomyelitis reported in Medellín

With respect to the Department, in general, the poliomyelitis cases were as follows

TABLE 5. REPORTED CASES OF PARALYTIC POLIOMYELITIS IN MEDELLÍN AND IN THE DEPARTMENT OF ANTIOQUIA DURING THE LAST SIX YEARS

YEAR	MEDELLÍN	TOTAL NUMBER OF CASES IN THE REST OF THE DEPARTMENT
1954	18	18
1955	12	13
1956	52	17
1957	18	23
1958	19	40
1959*	4*	7*

* Up to 17 June 1959.

Discussion

The results obtained up to the present (17 June 1959) have been described. We do not pretend to have immunized one hundred per cent of the population, neither do we believe we have the answers to solve all questions We do believe, however, that the childhood population of Medellín is now better protected against infantile paralysis than it was before the vaccination. This is what we wanted to achieve. The figures we have presented support this statement up to now and we trust with confidence that they will continue to do so in the future

Epidemiological supervision of all proved or suspect cases of poliomyelitis that may appear henceforward in the city of Medellín will continue

I shall at this point ask Dr. Martins da Silva to explain the distribution of antibodies in sample specimens before and after vaccination in Medellín in 1959

DR MARTINS DA SILVA · Table 1 * shows a two-dilution survey, 1 4 and 1 8, of naturally occurring antibodies in the area, a total of 1,047 specimens, including 133 specimens listed as adult but representing cord blood

I think the point to make here is the percentage of children with antibodies to all three types with increasing age. 11 per cent at one year of age, 14 per cent at 2, 35 per cent at 3, and so on up to 82 per cent in the 10-15 year old group.

* See p 459

The percentage of triple negatives is listed in a similar but inverse faction: 39 per cent at 1 year, 13 per cent at 2 year; 6 per cent at 3 year and 0 per cent in the 10-15 year old group

Table 2 † shows the antibody response to the vaccine based on 673 paired specimens In the group lacking antibodies before vaccination, the response to Type 1 virus was 88 per cent; to Type 2, 46 per cent; and to Type 3, 88 per cent

Figure 1 * shows the percentage of negatives, by age, before and after vaccination by virus type. It can be seen that the percentage of negatives after vaccination was considerably reduced for Types 1 and 3 and less so for Type 2

DR ABAD GÓMEZ: In closing I can say, as a public health officer, that I have the absolute conviction that the child population in Andes and Medellin is now better immunized, and that there are fewer cases of poliomyelitis, also, that we are substituting a wild virus for a much better one

During the course of this Conference, I have been torn between the idea of what is right and what is wrong in this field

I am not convinced, by any means, that what we did in Colombia was necessarily the best thing to do, but I do think that we took the best possible way open to us, other than doing nothing at all

I think that this Conference has proved that there are better ways of doing things, and that we all should improve our methods

And for that purpose we are going to have to take chances of not doing everything always and at all times right

Decay has come when safety alone is put at a premium. Victory is not going to be given to us just for the asking Wisdom, alone, I believe, is not the answer As much as wisdom we need, at this time, courage and faith

† See p 460
* See p. 458

9. THE USE OF ATTENUATED POLIOVIRUS IN AN EPIDEMIC AREA

DR. MAURICIO MARTINS DA SILVA, M.D., MIGUEL LÓPEZ BERRIOS, M.D., AND JUAN JOSÉ ALCOCER, M.D.*

DR LÓPEZ BERRIOS *(presenting the paper)*

Scattered cases of paralytic poliomyelitis have been reported in Nicaragua for several years. The first recorded outbreak of the disease, however, was in 1938 when the attack rate was 6.6 per 100,000 population. Endemic cases have occurred throughout the year, but epidemic outbreaks have seemed to coincide with sharp seasonal changes such as the beginning of the rainy season or during drought spells. From 1938 to 1957, the number of cases registered annually has varied from a low of 8 in 1947 and 1949 to a high of 191 in 1953. In the past 5 years the disease has appeared with increasing frequency and intensity; 1953, 1955, and 1958 being the peak years during this period.[1]

Poliomyelitis reporting in Nicaragua has always been confined to the paralytic form of the disease, and diagnosis is based only on clinical findings. Managua, the capital of Nicaragua, with a population of approximately 172,000 representing 12.5 per cent of the total of the country,[2] has contributed the largest number of cases in any one year. Table I shows the incidence of paralytic poliomyelitis in Managua and the rest of the country during the past several years.

* Dr Martins da Silva (Pan American Sanitary Bureau/World Health Organization), Dr Miguel López Berrios (Ministry of Public Health, Managua, Nicaragua), and Dr Alcocer (Pan American Sanitary Bureau/World Health Organization). (This paper has been accepted for publication by *The American Journal of Hygiene*.)

TABLE I REPORTED CASES OF PARALYTIC POLIOMYELITIS AND ATTACK RATES FOR THE REPUBLIC OF NICARAGUA AND THE DEPARTMENT OF MANAGUA—1951-1958

YEARS	NICARAGUA		MANAGUA	
	CASES	RATES†	CASES	RATES†
1951	32	2.9	22	11.4
1952	24	2.1	20	10.1
1953	191	16.4	99	48.3
1954	45	3.7	28	13.2
1955	113	9.1	69	31.5
1956	48	3.7	36	15.9
1957	68	5.1	34	14.5
1958	254	18.4	107	44.1

† Per 100,000 population
NOTE: Population of Nicaragua (estimated 1958—1,377,599)
Population of Managua (estimated 1958—242,443)

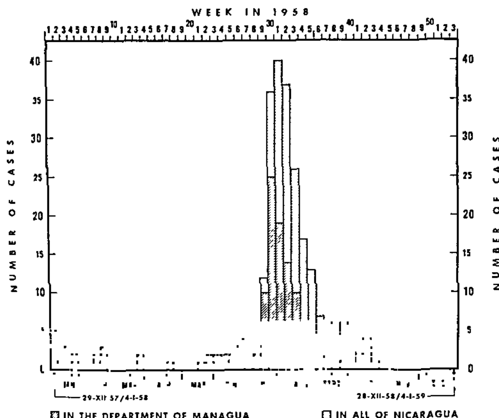


FIG. 1 Notified cases of paralytic poliomyelitis by week of onset in the department of Managua and all of Nicaragua—1958

In 1958, 254 paralytic cases of poliomyelitis, including 18 deaths, were reported in Nicaragua (Fig 1). The epidemic started on the week ending 26 May, reached a peak on the week of 2 August, and terminated on the week of 1 November. It lasted 22 weeks during which time 238 cases including 17 deaths were recorded (Table 2) Fourteen of the 16 Departments (states) of the country were affected (Figs. 2 and 3) Seventy-four per cent of the cases in the country and 85 per cent in Managua were in children less than 2 years of age (Table 3). Only 3 cases occurred in persons over 10 years of age, one of these, in Managua, was a 23 year-old foreign born female who was pregnant when she developed the disease

Following a request for assistance from the Ministry of Public Health of Nicaragua to the Pan American Sanitary Bureau, one of the authors (M M S) went to Managua in early August of 1958 to collaborate with the health authorities in the planning of a poliomyelitis vaccination program

During 11-13 August, stool and blood samples were collected from 21 recent paralytic cases in the low age group, and from 14 household contacts, the material was shipped under refrigeration to the Viral and Rickettsial Research Laboratory of the American Cyanamid Company in Pearl River, New York, and from there portions of these specimens were forwarded to the WHO Regional Poliomyelitis Laboratory at Yale Uni-

9. THE USE OF ATTENUATED POLIOVIRUS IN AN EPIDEMIC AREA

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YEARS	NICARAGUA		MANAGUA	
	CASES	RATES†	CASES	RATES†
1951	32	2 9	22	11 4
1952	24	2 1	20	10 1
1953	191	16 4	99	48 3
1954	45	3.7	28	13 2
1955	113	9 1	69	31 5
1956	48	3 7	36	15 9
1957	68	5 1	34	14 5
1958	254	18 4	107	44 1

† Per 100,000 population

NOTE: Population of Nicaragua (estimated 1958—1,377,599)
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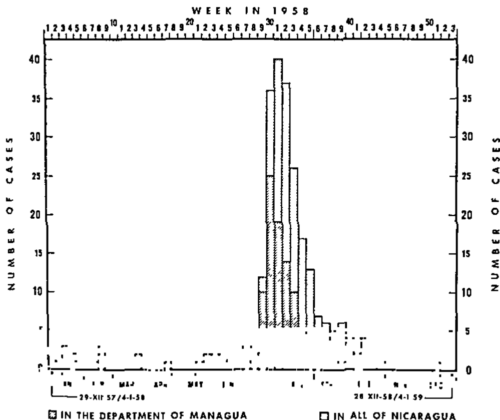


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Following a request for assistance from the Ministry of Public Health of Nicaragua to the Pan American Sanitary Bureau, one of the authors (M M S) went to Managua in early August of 1958 to collaborate with the health authorities in the planning of a poliomyelitis vaccination program.

During 11-13 August, stool and blood samples were collected from 21 recent paralytic cases in the low age group, and from 14 household con tacts, the material was shipped under refrigeration to the Viral and Rickettsial Research Laboratory of the American Cyanamid Company in Pearl River, New York, and from there portions of these specimens were forwarded to the WHO Regional Poliomyelitis Laboratory at Yale Uni-

RIO SAN JUAN
JINOTEGA
MANAGUA
RIVAS
MASAYA
LEON
CHINANDEGA
GRANADA
ZELAYA
CARAZO
ESTELI
BOACO
CHONTALES
MATAGALPA

ONE CASE

WEEK IN 1958

Fig 2 Notified cases of *paralytic poliomyelitis in Nicaragua by departments and by week—1958*

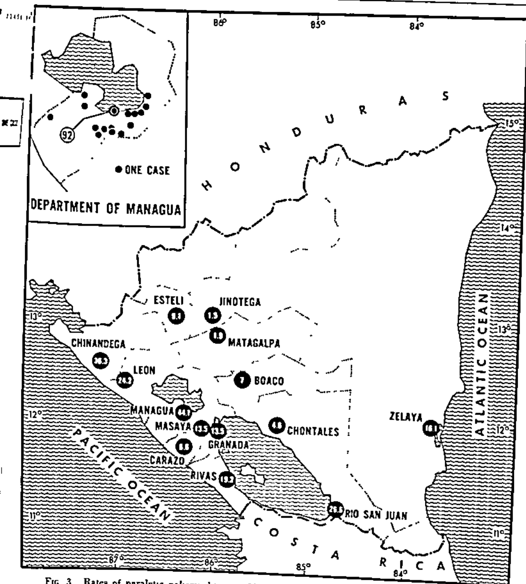


FIG 3 Rates of paralytic poliomyelitis per 100,000 population by departments in Nicaragua, and distribution of paralytic cases in the department of Managua—1958

versity for parallel diagnostic examination Type 2 poliovirus was isolated in one laboratory or the other, and usually in both, from the stools of 19 cases and 4 household contacts. In the 2 cases in which no poliovirus was isolated from the stools, serum antibodies were detected only for Type 2 poliovirus.

Of the 23 specimens yielding Type 2 virus, 13 came from children who were one year of age or less More than half of those harboring Type 2 [illegible]

[illegible] Type 2 virus. The antibody levels for Type 2 virus in the other

TABLE 2. REPORTED CASES OF PARALYTIC POLIOMYELITIS IN NICARAGUA, 1958

PERIOD	CASES	DEPARTMENT OF MANAGUA	OTHER DEPARTMENTS
January—May	17	9	8
Epidemic Period			
June	11	7	4
July	76	48	28
August	110	39	71
September	25	2	23
October	14	2	12
November—December	1	0	1
Total	254	107	147

TABLE 3 REPORTED CASES OF PARALYTIC POLIOMYELITIS BY AGE GROUPS IN NICARAGUA, 1958

AGE GROUP	CASES	DEPARTMENT OF MANAGUA	OTHER DEPARTMENTS
Total	254	107	147
Less than 1 year	102	50	52
1 year	87	41	46
2 years	28	8	20
3 years	10	1	9
4 years	10	4	6
5—9 years	14	2	12
10—14 years	1	0	1
15—19 years	1	0	1
20—29 years	1	1	0
30 or more years	0	0	0

Cases under 3 years of age=86.8 per cent
Cases under 10 years of age=98.8 per cent

22 sera ranged from 1:16 to 1:1024. No other poliovirus type was isolated from the 32 stool specimens examined. In October, Type 2 poliovirus was isolated from the stools of 3 acute paralytic cases reported from Masatepe, León and Bluefields, three cities located in widely separated parts of the country. It was thus established that the epidemic was due to Type 2 poliovirus.

Organization and Conduct of the Vaccination Program

In consultation with the national and municipal health authorities, a plan of operation was developed for the rapid vaccination in Managua of all children between 2 months and 10 years of age. A live attenuated poliovirus vaccine was selected on the basis of previous experience with

this type of vaccine in Andes, Colombia, earlier in the year[8]

For administrative purposes, the city of Managua was divided into 7 zones, each of which was subdivided into 7 sectors Each zone was placed under the supervision of a graduate nurse who directed the activities of the vaccination teams assigned to each sector The control of the 49 teams was under a graduate public health nurse and the over all program under the direction of a full-time epidemiologist. The actual administration of the vaccine was effected by practical and student nurses who were accompanied by sanitary inspectors acting as recorders

Separate family cards were filled out with each household member listed by name, age, sex, and Salk vaccine status. Each family was assigned a serial number within the zone and sector of residence, and the dwelling was appraised and rated as to its sanitary level A separate card was also prepared for each vaccinated child which included notations of age, sex, parents' names, and dates of vaccine administration and collection of blood samples

The vaccines employed were fluid preparations made from the same virus strains as those described by Cabasso *et al.*,[4] each virus type was administered separately in 0 5 ml doses from dropper bottles by means of a graduated glass tube and rubber bulb The dose was measured into a disposable plastic spoon and given by mouth The virus content of each of the three type-specific vaccines was Type 2, $10^{6.0}$ TCD_{50}; Type 3, $10^{5.2}$ TCD_{50}; and Type 1, $10^{6.7}$ TCD_{50}

The vaccination teams made at least three visits to each household within the sector; they administered the vaccine and posted the individual and family cards at the time of their first visit Pertinent notes concerning intervening illnesses or reactions following vaccination were entered on each individual card

Blood samples were collected from vaccinated children at 2 fixed stations within the city of Managua and by a mobile unit in the rural areas *Administration of Type 2 vaccine in the city of* Managua started on 5 September 1958 and primary coverage was completed 12 days later During this time 42,199 children between the ages of 2 months and 10 years were vaccinated They represented 80 per cent of the estimated population under 10 years of age. Completion of the Type 2 feeding in Managua was followed by the administration of Types 3 and 1, in that order A period of 3 weeks elapsed between each of the three separate feedings

Immediately following the conclusion of the Type 2 feeding in the city of Managua, the program was extended to the surrounding rural *area where 30 small communities in the Depart*ment of Managua were organized into an eighth administrative zone Due to the sparseness of the population in these outlying areas, vaccination was carried out, in many instances, by congregating the population in churches or schools where vaccination stations were set up In response to requests from the National Government and the local authorities in Corinto and Rivas, two small communities in the western part of Nicaragua, vaccination teams were assigned to these villages where a total of 6,575 children received all three virus strains.

After the initial and major part of the vaccination project had been completed in the urban and rural areas, a maintenance program was instituted in Managua for vaccination of newborn infants at the maternity services of the General Hospital, the Social Security Institute, and the 5 Municipal Health Centers Children who were missed by these centers, received the vaccine at a special station set up at the Baptismal Service of the Catholic Cathedral Vaccination of newborns is being done by the simultaneous feeding of all 3 strains of virus Some of these infants have received a 1 5 ml feeding consisting of 0 5 ml of each of the 3 vaccines. At present, infants receive 2 0 ml of a trivalent preparation which contains $10^{6.3}$ TCD_{50} of each virus type As of 15 May 1959, a total of 2,418 newborn infants had been vaccinated under the maintenance program

On the basis of demographic data it was estimated that the population under 10 years of age, at the outset of the program, in the city of Managua was 52,600, and 9,452 in the immediate surrounding area In the city of Managua, between 5 September 1958 and 15 May 1959, 98 per cent of these children received Type 2 vaccine, 91 per cent Type 3, and 82 per cent Type 1 The corresponding rates in the surrounding

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Cases under 3 years of age=86 8 per cent.
Cases under 10 years of age=98.8 per cent

22 sera ranged from 1:16 to 1:1024 No other poliovirus type *was isolated from the 32* stool specimens examined. In October, Type 2 poliovirus was isolated from the stools of 3 acute paralytic cases reported from Masatepe, León and Bluefields, three cities located in widely separated parts of the country. It was thus established that the epidemic was due to Type 2 poliovirus

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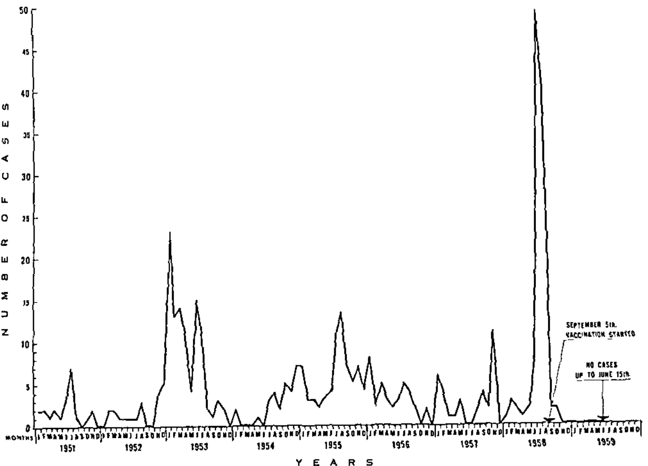


Fig 4 Reported cases of paralytic poliomyelitis in the department of Managua, by month 1951 to 1959

rural sectors were 85 per cent for Type 2, 74 per cent for Type 3, and 65 per cent for Type 1. The transient nature of the working population which is seasonally engaged in the harvesting of coffee and cotton was a major factor in reducing the number of those who received all three types of the vaccine It was not uncommon for vaccinating teams to find unoccupied homes where cooperating families had been registered previously

Of the children who had received all three strains of oral vaccine, 6 4 per cent had had one injection of Salk vaccine during 1958, 2.3 per cent had received 2 injections, and 0 9 per cent had had 3 injections

As of 15 May 1959, a total of 59,855 children had received Type 2 virus, 54,732 Type 3, and 49,585 all three types of poliovirus oral vaccine in the Department of Managua

Epidemiological Observations

The vaccination campaign on the whole was well received by the people of Managua There was some early but short-lived disapproval voiced by the press and radio The net result of this disapproval was perhaps to increase in some degree the thoroughness of surveillance and to bring larger numbers of children to the campaign headquarters with post vaccination complaints Among the 225 children examined there by the medical staff, 91 per cent were found to be suffering from common respiratory or gastrointestinal complaints, and the remainder from miscellane ous infections including measles, mumps, pertussis, and streptococcal pharyngitis There were four instances of urticaria, one of which was moderately severe occurring in a family of six vaccinated children, the eruption manifested itself shortly after administration of the Type 1 vaccine. The symptoms subsided rapidly under treatment and none of the other children in the same family experienced any discomfort or difficulty.

After the oral vaccination program was started in Managua, 3 cases of paralytic poliomyelitis were reported within the area of operation, but none of them in vaccinated children or contacts thereof. Of these, one occurred in a child whose parents refused vaccine, and the other two were early in the program in outlying sectors where vaccination had not yet commenced One of these cases developed in September and the other 2 in the second week of October. During the succeeding 8 months, i e. from 15 October 1958 through 15 June 1959, there have been no further cases of paralytic poliomyelitis reported in Managua (Fig 4)

Serologic Observations

To ascertain the antibody status of the population to be vaccinated, serum specimens were collected from 505 children whose ages ranged from 6 months to 10 years immediately before the administration of Type 2 virus The methods employed for titration of serum antibody are described elsewhere [5] The distribution of neutralizing antibodies and the percentage of negatives for each of the three types of poliovirus is indicated by age in Table 4, where it may be seen that only 8 per cent were without Type 2 antibody and that approximately 20 per cent of the entire group lacked antibodies for Type 1 and Type 3 polioviruses It may also be noted that the great majority of the susceptible children were under 2 years of age and that even at this early age only 28 of 120 were without antibody for Type 2 poliovirus This is doubtless a reflection of the prevalence of this virus type in the community Fig. 5, based on the geometric mean antibody levels for the 3 virus types by age, is indicative of the recent poliovirus activity in Managua The distribution of seronegatives by virus type and combinations is summarized in Tables 5 and 6 These data, as well as those in Table 4, indicate the tempo of poliovirus dissemination in the study area and suggest that the prevalent Type 2 virus had already infiltrated the population very thor oughly before control measures were undertaken

It was the original intent to obtain paired sera from approximately one per cent of the children vaccinated However, owing to the mobility of the working families in Managua, it was impossible to obtain post vaccination specimens from more than approximately one half of the children from whom sera were obtained at the time of the first feeding. Of the 505 children bled at the time of the initial feeding, post vaccination specimens were collected from 242 The distribution of seronegatives among these paired sera is shown in Tables 5 and 6, where it may be seen that 82

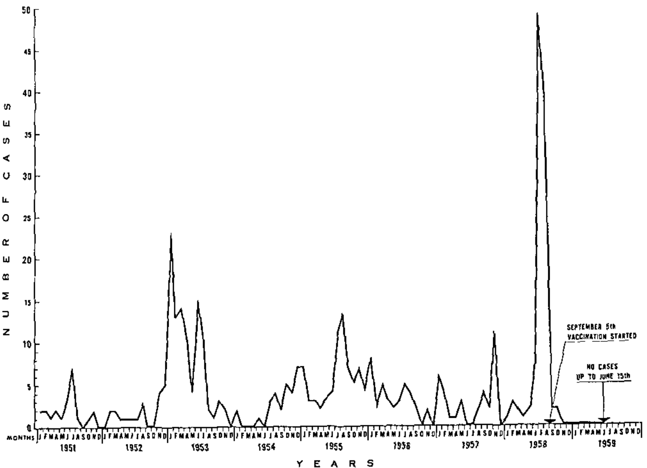


Fig 4 Reported cases of paralytic poliomyelitis in the department of Managua, by month 1951 to 1959

TABLE 4 DISTRIBUTION OF NATURALLY OCCURRING POLIOVIRUS ANTIBODIES AND PERCENTAGE OF NEGATIVES BY IMMUNOTYPE AND AGE AMONG 505 CHILDREN—MANAGUA, NICARAGUA, 1958

AGE IN YEARS	NUMBER OF CHILDREN	TYPE 1		TYPE 2		TYPE 3	
		NUMBER POSITIVE	PERCENTAGE NEGATIVE	NUMBER POSITIVE	PERCENTAGE NEGATIVE	NUMBER POSITIVE	PERCENTAGE NEGATIVE
< 1	48 (9)*	11	76	34	29	14	71
1	72 (5)*	40	44	59	18	36	50
2	69	53	23	64	7	57	17
3	81 (1)*	70	14	77	5	73	10
4	73	68	7	73	0	70	4
5	29	29	0	27	7	29	0
6	27	27	0	25	7	26	4
7	38	38	0	37	3	36	5
8	34	34	0	34	0	33	3
9	28	26	7	27	4	27	4
10	4	4	0	4	0	4	0
<10†	2	1	50	2	0	2	0
TOTAL	505 (15)*	401	21	463	8	407	19

* ( )—Number of triple negatives in group
† —Age data not recorded but less than 10 years of age

TABLE 5 DISTRIBUTION OF POLIOVIRUS ANTIBODY SERONEGATIVES AMONG 242 CHILDREN BEFORE AND AFTER LIVE ATTENUATED POLIOVIRUS VACCINATION BY AGE AND BY SEROTYPE—MANAGUA, NICARAGUA—1958

AGE IN YEARS	ANTIBODY NEGATIVES FOR					
	TYPE 1		TYPE 2		TYPE 3	
	PRE	POST	PRE	POST	PRE	POST
<1	18	3	6	4	16	4
1	15	6	8	3	20	5
2	12	3	4	1	5	1
3	4	1	0	0	4	0
4	2	1	0	0	1	0
5—10	2	0	1	0	4	0
Total*	53	14	19	8	50	10

* Ninety of 122 negatives, or 74 percent, responded to vaccination, Type 1 negatives 53/242=22%, Type 2 negatives 19/242=8%, Type 3 negatives 50/242=21%.

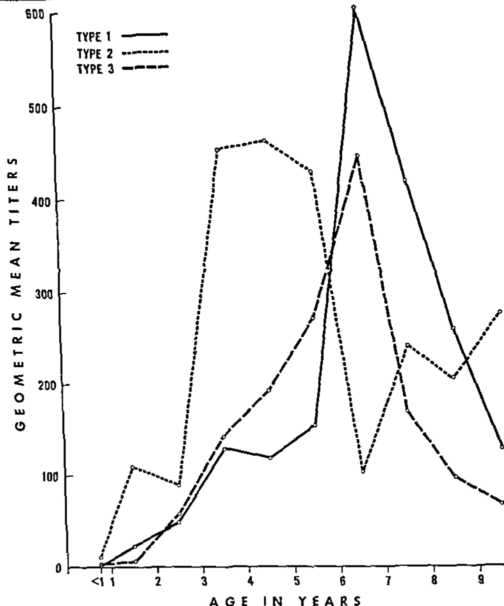


FIG 5 Geometric mean titers of poliovirus antibodies by immunotype and age before oral vaccination—Managua, Nicaragua, 1958

of the 242 children were lacking in antibody for one or more types of poliovirus Seven individuals were triple and 26 were double antibody negatives Approximately 85 per cent (104/122) of the 122 pre vaccination seronegatives are found in children under 3 years of age, Type 1 antibody was lacking in 22 per cent of the 242 children, 8 per cent were without demonstrable antibody for Type 2, and 21 per cent for Type 3 By applying these percentages of type-specific

TABLE 6 DISTRIBUTION OF SERONEGATIVES, BEFORE VACCINATION, AMONG 505 CHILDREN, BY AGE, VIRUS TYPE AND COMBINATIONS—MANAGUA, NICARAGUA—1958

AGE IN YEARS	NUMBER OF CHILDREN	SERONEGATIVES BY VIRUS-TYPE AND COMBINATIONS							POSITIVES TO ALL 3 TYPES
		1	2	3	1—2	1—3	2—3	1—2—3	
<1	48	5	0	5	4	19	1	9	5
1	72	10	4	16	3	13	2	5	19
2	69	12	3	7	1	3	2	0	41
3	81	9	1	7	1	0	0	1	62
4	73	4	0	2	0	1	0	0	66
5—10*	162	5	5	4	1	1	1	0	145
Total	505	45	13	41	10	37	6	15	342
Paired Sera†	242	22	6	21	4	20	2	7	160

* Includes 2 specimens from children under 10 years but without further age specification

† These sera represent the number of pre-vaccination samples for which a paired specimen was obtained after vaccination

negatives in the sampled population to the numbers of children vaccinated with each of the 3 virus strains, it may be calculated that there were approximately 11,000 children who were lacking in demonstrable antibody for Type 1 poliovirus, 4,800 who were negative for Type 2, and 11,550 negative for Type 3 at the time of vaccination

The antibody responses to each of the 3 virus types are summarized in Table 7, in terms of the numbers and percentages of children who showed four-fold or greater increases in antibody titer after oral vaccination Of those who had no homologous antibody at the time of vaccination, 74 per cent responded to Type 1 poliovirus, 58 per cent to Type 2, and 80 per cent to Type 3

DISCUSSION

Several aspects of the experience with oral poliovirus vaccine in Managua deserve comment

Safety of the Vaccine With the exception of the four cases of urticaria, only one of which developed shortly after ingestion of the Type 1 strain, there were no indications that the administration of the vaccine was causally related to illness in the children. Since urticaria has only rarely been noted elsewhere among the great numbers that have received these vaccines, it is unlikely that the cases reported were, in fact, due to the ingestion of the virus preparations used There were clinical complaints registered, but when investigated by the medical staff they were found to be of the usual type expected in a childhood population having the age composition and size of that involved in the vaccination campaign It cannot be assumed that all instances of clinical illness following vaccination were reported, however, none of the data accumulated during or since the feeding program suggests that there *is reason to doubt the safety of the oral vaccines* employed in Managua

Ease of administration From the standpoint of the public health administrator, the experience in Managua demonstrated that the oral vaccine has marked advantages. Chief among these is the fact that it was possible, without the difficulties and delays involved in assembling a large and skilled medical staff, to utilize the facilities and the personnel at hand in carrying out the actual

TABLE 7 ANTIBODY RESPONSE OF VACCINATED CHILDREN BY IMMUNOTYPE—MANAGUA, NICARAGUA—1958

	TYPE 1			TYPE 2			TYPE 3		
		Positive Responses 4x or Greater			Positive Responses 4x or Greater			Positive Responses 4x or Greater	
Pre-Feeding Titers	Total Number Fed	Number	Per Cent	Total Number Fed	Number	Per Cent	Total Number Fed	Number	Per Cent
<4	53	39	74	19	11	58	50	40	80
4	10	8	80	14	10	71	20	16	80
16	30	23	77	18	14	78	33	26	79
64	45	10	22	46	26	57	50	27	54
256	53	8	15	93	41	44	55	19	35
Sub-Total	191	88	46	190	102	54	208	128	62
≤1024	51	—	—	52	—	—	34	—	—
Total	242	88	46	242	102	54	242	128	62

vaccination program, that this was done rapidly and effectively is indicated by the fact that 12 days after the initiation of the Type 2 feeding in Managua, 42,199 children had been vaccinated with the type of vaccine corresponding to the epidemic strain Operations in the rural areas proceeded of necessity, at a slower pace due to limited transportation facilities and time lost in travel The ready acceptance of the oral mode of vaccination on the part of the public was a factor of importance in contributing to the ease and rapidity with which the work was done It was the collection of blood samples rather than the administration of the vaccine that met with resistance With the availability for mass application of a trivalent vaccine such as that now being used in the maintenance program, the Managua campaign could be carried out in even less time and at considerably less cost Of great importance, too, is the fact that with the use of the trivalent vaccine there would be no diagnostic delay required to determine the epidemic type of virus before starting a control program

The serologic evidence developed during the Managua study provides data which in addition to their value in determining the response to oral vaccination are of interest in speculating about the genesis and dynamics of the epidemic First, attention may be directed to Fig 5 in which the prevaccination serologic survey data are summarized It may be noted that the geometric mean titer for Type 2 antibody rises sharply at the 2 year level and then drops markedly at about 6 years of age Coincident with this drop in Type 2 titers, there is a pronounced rise in mean titer of the Type 1 antibody It seems unlikely that the children over 5 years of age were less exposed to the Type 2 epidemic strain than were the younger children. It will be recalled, however, that the 6-year-old group of 1958 was in the 1-year category in 1953 when Managua had 99 cases of paralytic poliomyelitis Information regarding the type of poliovirus responsible for the 1953 epidemic is not available, but the present serologic data suggest that it may have been a Type 1 or a mixed Types 1 and 3 outbreak The

unusually high levels of the Type 1 antibody of the 6-year old children, in contrast to the levels for Type 2, suggests that exposure to the antigenically broader Type 2 virus of 1958 resulted in a greater serologic response to the primary infecting strain or strains of 1953 than to the current Type 2 virus

Second, the prevaccination serologic data afford a basis for reconstructing a tentative picture of the conditions which existed at the beginning of the 1958 epidemic in Managua In this outbreak, as had been true in the past, the great majority of the paralytic cases occurred in children under 2 years of age

The prevaccination survey (Table 6) showed that in early September, when the epidemic had largely subsided, there were still 12 per cent (14/120) of the children in this age group who were without demonstrable antibody to any of the 3 types of poliovirus Furthermore, 27 per cent (32/120) of the children in this age category were seronegatives for Type 1 and Type 3 virus, and it may be reasonably assumed that at the beginning of the epidemic these children, too, were triple negatives; thus, 39 per cent of the children under 2 years of age in Managua when the outbreak started were presumably without prior experience with any type of poliovirus Of the 120 children under 2 years of age included in the prevaccination survey, there were 28 who were lacking in antibody for Type 2 poliovirus If to this number be added the 32 who were positive only for Type 2, it appears that when the epidemic started, 50 per cent of the segment of the population which accounts for most of the paralytic poliomyelitis were presumably susceptible to the epidemic strain, and that when the epidemic subsided this proportion had been reduced to 23 per cent (28/120) in early September at the onset of the mass vaccination program If this calculation—that 50 per cent of the children under 2 years of age were susceptible to Type 2 poliovirus at the onset of the epidemic—is valid, it is a relatively simple matter to determine the paralytic attack rate during the 1958 epidemic. The Ministry of Health data (2) show that 20 7 per cent of the 74,188 children under 10 years of age in the *Department of Managua* [illegible]

gory in 1958 [illegible]

mately 7,700, may be assumed to have been Type 2 susceptible at the beginning of the epidemic In our sample of 120 children under 2 years of age, there were an estimated 60 (50 per cent) who were Type 2 susceptible at the outset of the epidemic. Twenty-eight or 47 per cent of these were still without Type 2 antibody when the survey was made after the outbreak was over; thus, 53 per cent became infected during the intervening weeks By applying these ratios to the 7,700 Type 2 susceptible children in Managua, it can be determined that 4,081 children (53 per cent) under 2 years of age became infected, and 91 of them developed paralytic poliomyelitis *Calculated on this basis, there were 45 poliovirus* infections for each paralytic case.

The evidence indicates that the outbreak was essentially at an end when the vaccination campaign was started This is shown by the reporting of only 3 cases of paralytic poliomyelitis after the vaccine feeding began, and also by the *serologic studies which demonstrated that the frequency* of Type 2 antibodies in infants and very young children was already at a high level, as compared to the rates for the other 2 types of poliovirus (Table 4).

The vaccination program was not without effect, however This may be seen from an inspection of the post-vaccination serologic data.

Of the Type 2 seronegatives among the 120 children under 2 years of age in the prevaccination survey, 14 appeared among the 55 from whom post-vaccination serum samples were collected (Table 5) Although only 7 of these 14 children responded serologically after vaccination, this rate of response reduced the proportion of susceptibles in this age category to 13 per cent in the post vaccination period Similarly, the Type 1 and Type 3 seronegatives in this epidemiologically important age group were reduced from 60 and 65 per cent, respectively, to 16 per cent after oral vaccination.

The vigorous maintenance program of vaccination in Managua during the post epidemic period of 8 months has apparently kept the proportion of susceptible infants and young children at effectively low levels As may be seen in Figure 4, during the seven years from 1951 through 1957, a total of 84 months, there have been only 15 scattered months in which no paralytic polio-

myelitis was reported The longest interval without reporting was a 90 day period following the 1953 epidemic Eight consecutive months without a reported case of poliomyelitis in Managua is unparalleled in the records

In the light of subsequent experience,* it appears that the vaccine preparations used in Managua were of less than optimum virus content to obtain the best ratio of response to all three types of poliovirus The data indicating the extent of poliovirus dissemination in the study area also suggest that the prevalence of other enteric agents, including potentially interfering viruses, would be equally high in the community and could, therefore, have been partly responsible for the lower ratio of response observed However, viral studies in stool samples to verify this hypothesis were not carried out

SUMMARY

An epidemic of paralytic poliomyelitis in which there were 254 cases and 18 deaths in Nicaragua was identified as a Type 2 outbreak

An oral poliovirus vaccination program was organized and carried out late in the epidemic in the city and department of Managua Type 2 poliovirus vaccine was administered to 42,199 children less than 10 years of age in a period of 12 days, in a house-to-house campaign Type 3 and Type 1 vaccines were subsequently fed at intervals of 3 weeks in similar fashion Of those who had no homologous antibody at the time of vaccination, 74 per cent responded to Type 1 poliovirus, 58 per cent to Type 2, and 80 per cent to Type 3 The mass vaccination program has been followed by a maintenance program in which newborn children are vaccinated at hospitals and health centers with a trivalent oral vaccine Since 5 September 1958, 59,855 children have received Type 2 vaccine, 54,732 were fed Type 3, and 49 585 have had all three types of oral poliovirus vaccine This represents a coverage of approximately 98 per cent, 91 per cent, and 82 per cent, respectively, of the eligible population in Managua, under 10 years of age Included among those vaccinated with all three virus types were 2,418 newborn infants who received the trivalent vaccine

No illness attributable to the vaccine was observed and the vaccination program was well received by the population of Managua

Although the vaccination program was organized too late to affect the progress of the epidemic, its effect may be evident in the fact that there has been no paralytic poliomyelitis reported in Managua for 8 consecutive months since the completion of the mass vaccination program, a fact not observed in the preceding 8½ years *

The advantages of oral poliovirus vaccine are discussed from the viewpoint of the public health administrator, and the dynamics of the epidemic are considered

ACKNOWLEDGMENTS

The attenuated poliovirus vaccine strains used in the program—Type 1, SM; Type 2, MEF₁, Type 3, FOX—were provided by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N Y, through an agreement with the Pan American Sanitary Bureau.

Dr Herald R Cox and staff carried out the virus isolation and antibody titrations reported

The authors wish to thank Drs Floyd S Markham and John A Kerr for the helpful suggestions given in the preparation of this manuscript

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* The period without reported cases of paralytic poliomyelitis in the Department of Managua has now been extended to 27 August 1959, a total of 10½ months

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DISCUSSION

CHAIRMAN RHODES: The papers presented by Dr Abad Gómez and by Dr López Berrios are open for discussion

DR COX I wish first to congratulate both Dr. Abad Gómez and Dr. López Berrios on their excellent presentations As a matter of fact, I did not know the final outcome in Nicaragua until today. May I discuss both papers at the same time?

As Dr Abad Gómez points out, there is no doubt that the Type 1 poliovirus encountered in Andes must have been a highly virulent type because, as I told you the other day, a strain of virus was recovered from a Colombian girl who had been paralyzed in both legs for two months—perhaps permanently Her stool samples contained 50 TCD_{50} of Type 1 virus per gram of feces The first tissue culture passage of the virus contained 200,000,000 TCD_{50} per ml This first passage virus, when inoculated into monkeys in various dosages down to 20 TCD_{50} of virus, paralyzed practically every monkey, and one of two monkeys injected with 2 TCD_{50} The inoculations were done intracerebrally with half a cc dose unilaterally As a matter of fact, had I realized what we were working with, I suspect we would not have worked with the virus in our laboratory.

Now, it is important to point out here that only after the results came through did we realize that the serologic responses obtained in Andes were almost identical with those obtained in Minnesota, particularly the responses to Types 1 and 3 If you will recall, in Minnesota we fed Type 2 first, followed by Types 1 and 3 In Andes, we fed Type 1 first, followed by Types 2 and 3 The only strain that elicited a response rate of less than 85 per cent was Type 2. We believe this may be partly explained by interference of the Type 1 virus in Andes

Regardless of what the monkey test shows in the laboratory, I still believe that you cannot put all your faith in such a test I think that in the long run, chances are better than even that this strain or Type 2 virus may turn out to be the most bona fide attenuated virus of the three types I say this because, in spite of the fact that pathologic lesions were found when this Type 2 virus was inoculated intracerebrally into monkeys, paralysis was rare. Furthermore, compared to other strains, this one has lost some of its infectivity for the human gut and is associated with a very short period of excretion We received 20 stools from Minnesota volunteers in the trial of 1958 and injected intracerebrally in monkeys as 20 per cent stool extract at 5 cc volumes per dose Not a single monkey showed any sign of disease My two grandsons, aged 3 and 1, also received this virus Of 6 monkeys inoculated with stool suspensions from these children, only one showed transient weakness Perhaps this is owing to the fact that this virus had been carried through 157 passages in suckling hamsters I do not know how many generations in mice, and into the chick embryo You cannot carry out such a procedure without altering the characteristics of a virus, I assure you

The interesting thing is that we did not get any evidence that Types 1 and 3 were being interfered with by enteroviruses I say this because we have all been concerned on this score and I wish to remind you that it is possible to over modify a virus to the point where the virus may be safe but it also may no longer be immunogenic for man I think I need only point to high-passage yellow fever virus in this respect After all, I think we all agree that we are here to immunize people and not to protect monkeys We must have a virus that immunizes as well as one that is safe.

From Nicaragua we received something like 37 bloods and stools, and in our laboratory we *were only able to make 11 Type 2 isolates These* were isolated and identified by the third day after receipt We did not believe our results at first, and it was only by the fifth day, when the results of the serum samples came through, confirming the fact that they were Type 2 and that there were no Type 1 antibodies in the bloods, that we called Dr Soper and told him we were dealing with Type 2 isolates.

These same stools were split and sent on to Dr. Paul's laboratory on the same day we received them and, roughly four or five weeks later, Dr Paul reported that he had also found Type 2 Incidentally, he found 22 Type 2 isolates whereas we found only 11 We have since checked with Dr Melnick to find out what technique we might use to increase the sensitivity We have re checked the stools twice, using HeLa and primary monkey kidney cells, but still have found only 11 isolates We are now going to try using human amnion cells.

Now, as you know, we really did stick our neck out with the Type 1 vaccine I was not worried about Type 2 because it is a unique strain, in that we have two markers to identify it. This virus is highly active in the mouse and hamster, and furthermore, despite continued laboratory production in monkey kidney tissue culture, it still retains the ability to grow in chick embryo culture even after recovery from the human stool

Actually, the mouse marker is so strong that we have to block our Type 2 poliovirus with a Type 2 antiserum in order to check for Coxsackie and other virus contaminants Sometimes we wish we had never marked the virus so thoroughly, because it makes laboratory testing more difficult when we try to eliminate simian viruses

I am convinced that we were just lucky in a certain sense with the viruses we used Dr Sabin pointed out yesterday that when you try to break an epidemic you do not usually send a boy to do a man's work It is necessary to feed the vaccine as fast as possible and you want to be sure that you are feeding an adequate quantity of virus—and the dosage might differ with the strain of virus involved We believe that our Type 2 is not as invasive, and we do not get the immunogenic response with it, at the same dosage level, that we get with Types 1 and 3

Actually, I think our Type 3, which is the only strain that has never been in any host other than man or monkey kidney, is possibly the most unaltered type, even though it seems to be the least reactive in the monkey virulence test This strain has never been adapted to rodents or chick embryos. These points are technical but are interesting in regard to basic research

The trials in Mana[illegible]re the first in which we were called up[illegible] in such haste, and we did not have enough virus-filled capsules on hand We also bore in mind the difficulty of feeding the capsules to babies during the Andes trial So, in the Managua emergency we decided to send a liquid preparation We had stock virus material on hand stored at minus 20.

I know I have an industrial competitor here in the audience, but he is also a fellow scientist and I think he will respect what I am saying We learned, the sad way, that you cannot harvest tissue culture material, store it at minus 20, and be sure of keeping it stable. This is particularly true of our Type 2 strain. We thought that the vaccine we sent to Managua had a titer of 10^5 to $10^{5.6}$ TCD_{50}—because we had used 12 tubes per dilution and 4 dilutions per type in calculating the 50 per cent end point—but after the vaccine was sent we discovered, much to our dismay, that the Type 2 vaccine actually contained only about $10^{4.3}$ TCD_{50} per ml The virus content had dropped one full log at minus 20, at a pH of 8.2, and it shows that, even though frozen, our Type 2 strain, at least, deteriorates We now think we have corrected that I am not going to say how, but I have to say my friend probably knows already.

We feel that we have learned—and from what we have shown on the board, I am sure you will agree—that virus dosage is very important, not only in assuring adequate immunization but probably also in combating the problem of enterovirus interference

We have decided that we are not going to send any vaccine out unless the dosage fed contains approximately 1,000,000 TCD_{50} of virus, and perhaps we should feed even more. It is interesting to know that in Managua they have continued to feed all newborns the trivalent material containing roughly three million tissue culture doses, which is the same material that has been fed in Minnesota and by Dr. Embil in Cuba If the dosage needs to be higher to assure a higher protection rate, that can be done, of course

We have titrated our material three consecutive times in two separate laboratories before it has been issued In other words, we actually get six titration assays on any vaccine lot, since we believe that it is most important to be able to back up the virus titer assays

The liquid preparation we are now working with looks most encouraging from the standpoint of stability Our data indicate that we can keep it at room temperature—which in the Pearl River Laboratories is about 76°F.—for about four to five weeks Stored at plus 4°C., the vaccine is stable for at least 8 months; even so, we still insist upon shipping the vaccine under refrigeration, particularly in shipments to the tropics, because we hesitate to allow it to stand under tropical airport conditions for 24 to 48 hours without refrigeration.

I think I have pointed out that under hurried conditions it is not always possible to send out the best product We do wish to send out our best product These are the only comments I wished to make.

CHAIRMAN RHODES: Dr Sabin

DR SABIN I came in late, after this discussion started, but I did want to comment that I was very much impressed by one special point in the excellent presentation by Dr Berrios of the experience in Nicaragua, chiefly because I think it has something to tell us for the future

The most striking thing that I underline in his presentation was that this was a mass feeding, varying in different parts of the area from 80 to 97 per cent of the child population under consideration, and that the major portion of it was achieved within 12 days

I believe that the interpretation of all subsequent data that have been shown here, both the serological surveys and the clinical observations, must be viewed in the light of that, which I think is perhaps one of the most important indications of how things should be done in the future

Now, perhaps Dr Cox, my good friend and old associate over a period of 24 years, will permit me a comment on his statements regarding the Type 2 vaccine under consideration here

I think I need hardly stress it is a most interesting strain But also I would like to call attention to an observation that I made a number of years ago, and published about four years ago, that one of the things that happens to Type 2 virus after it is thoroughly adapted to mice is that it loses largely its capacity for multiplication in the intestinal tract as measured in monkeys

Now, those were experiments on cynomolgus monkeys, in which I compared the monkey spinal cord passaged virus with the same virus after a number of passages in mice; and although the material had the same—this was virulent virus—intracerebral activity in monkeys, the capacity to infect cynomolgus monkeys in the intestinal tract was markedly reduced in the mouse-adapted virus, and this was a fixed change, because subsequent passage back again into the monkey brain did not bring back the capacity to infect the intestinal tract.

Now, obviously, the Type 2 virus that Dr. Cox is using now, after further change in adaptation to monkey kidney tissue culture, is not quite the same as that originally used by passage in chick embryos And its behavior now, I think, is very much better in the intestinal tract than it was when the chick embryo material was used.

Before I go on with my other statements, may I make this statement that its behavior now is better than the chick embryo dose per dose.

DR. COX: I discussed these things before Dr. Sabin came in. I pointed out that our Type 2 strain poliovirus has the least infectivity for the human gut and shows the shortest period of excretion. Also, it apparently has the least spread, from what we know about it. Perhaps its slight spread may be considered as one of its deficiencies. This may be overcome by an increase in virus dosage, but that is one of our concerns

DR SABIN: My question was: Does the virus in the present form, in tests made in human beings, have greater activity in the intestinal tract than the same virus when it was in the chick embryo form?

DR COX: I believe that is true. The Type 2 strain put back into monkey kidney tissue culture seems to do a better job of immunization with *the same dosage level than it did* when grown in chick embryo. I believe we would be in full agreement on that point

DR SABIN: My point about that would be, therefore, that this present Type 2 virus is not the same virus as was the chick embryo material, and that the data included on this cannot in any way be correlated from the point of view

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DR. SABIN My point about that would be, therefore, that this present Type 2 virus *is not* the same virus as was the chick embryo material, and that the data included on this *cannot in* any way be correlated from the point of view

of characteristics similar to the original chick embryo virus.

Now, the point of short duration of multiplication and poor spread, and also the diminished antigenicity for antibody formation, are all related to a property which I think is highly desirable in an attenuated poliovirus vaccine, namely, that resistance of the intestinal tract is related to the extent of viral multiplication originally, it is desirable to have it multiply extensively, to have it spread extensively, because the better it multiplies in the intestinal tract, the better will be its antigenic response, and also the longer the duration of the response In quantitative studies with other strains, we have found that if you give a smaller dose, and the duration of multiplication is only for a limited period of time, when you merely look at the antibody four weeks later, it will seem to be all right But if you look at it two to three months later, you will find that it has disappeared

So, mere multiplication in itself is not an indication of full activity

Now, there is only one other point, and I think it is of importance Perhaps I misunderstood Dr Cox, but I got the impression that he suggested that there was no evidence that naturally occurring enteroviruses, that is, Coxsackie ECHO, interfered with the implantation and activity of the particular strains that he used

Now, before I go further, I would ask whether I am wrong in this assumption

Dr. Cox: All I can say is that when we analyze the immunogenic responses obtained in Minnesota and Andes to Types 1 and 3 polioviruses, they seem to be almost identical We know that in Colombia there must have been plenty of other viruses in the intestinal tracts of the vaccinated volunteers, and this poses a question· Are we actually dealing with interference by other viruses as much as some people think we are? As yet, we do not know the answer to this, but when you look at the record, the Minnesota and the Andes response data for Types 1 and 3 polioviruses are almost identical and the same vaccine materials were fed in both places.

Dr Sabin I am not disputing the fact that the data in Colombia and Minnesota are similar, but I think the interpretation that this means that there was no interference, or that there is no interference with these strains, cannot be made, because the data in Colombia represent feeding after a long period of time, a period which has permitted continued spread and reinfection of children who might have had interference

Furthermore, in the strains in which we were able to demonstrate interference, studies by Dr Ramos Alvarez—to be reported later—in the field over a period of about nine weeks showed exactly similar results

So one cannot use field data of this sort, based upon bleedings taken after a considerable number of weeks, to say whether or not there was interference, they merely show an end result

10. VIRAL AND SEROLOGICAL STUDIES IN CHILDREN IMMUNIZED WITH LIVE POLIOVIRUS VACCINE—PRELIMINARY REPORT OF A LARGE TRIAL CONDUCTED IN MEXICO

MANUEL RAMOS ALVAREZ, M.D., FEDERICO GÓMEZ SANTOS, M.D., LUIS RANGEL RIVERA, M.D., AND OTILA MAYES, Q.B.P.

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Mexico City, Mexico

DR RAMOS ALVAREZ *(presenting the paper)* We shall try to summarize in the first place some aspects of the natural history of poliomyelitis in Mexico during the last 10 years. Poliomyelitis is a disease of infants in Mexico, in contrast with the situation which is now observed in countries like the U.S.A., where a high percentage of cases is seen in older children and adults.

TABLE 1. AGE DISTRIBUTION AMONG 793 PARALYTIC CASES OF POLIOMYELITIS IN MEXICO (1954-1958)

AGE IN YEARS	NUMBER OF CASES	PER CENT OF CASES
<1	231	28
1—1 11	301	38
2—2 11	117	15
3—3 11	50	6
4—4 11	28	3
5—10	49	6
10	17	2
ALL	793	

Table 1 shows the age distribution of 793 paralytic cases of poliomyelitis observed at the Children's Hospital in Mexico, between 1954-1958. Although there are some cases in older children and sometimes even in adults, 81 per cent of those affected are under 3 years of age and 92 per cent are under 5 years of age.

Prior to 1946 only a few cases of poliomyelitis were reported and these were confined mostly to the Capital of the Republic. From 1946 to date, the number of reported cases has increased considerably. Figure 1 shows the number of cases reported from 1943 to 1958. It is interesting to note that major outbreaks of poliomyelitis occur in Mexico every two years.

The seasonal distribution of poliomyelitis in Mexico City from 1955 to May 1959 is shown in Figure 2. As can be seen from this figure the summer rises vary somewhat with the years, for example, in 1955 and 1959 the rise began as early as March, whereas in 1957 and 1958 it started as late as May or June.

Prevention of poliomyelitis is a major public health problem in Mexico. In order to obtain some information concerning the possibility of controlling the disease in the country with the use of a live poliomyelitis vaccine, we have carried out studies with this vaccine in an increasing number of children in a step wise fashion. The strains used in our studies were those developed by Dr Sabin, who made them available to us. They were aliquots of the large lots prepared by him in 1956 and used by different investigators on approximately 4.5 million people.

Preliminary studies were carried out in 1957 in an orphanage in Mexico City. These studies included an original group of 73 children under 3 years of age, and a group of 108 children of the same age who were fed various doses of virus separately or in mixtures. The results of these studies have been reported elsewhere. On 17

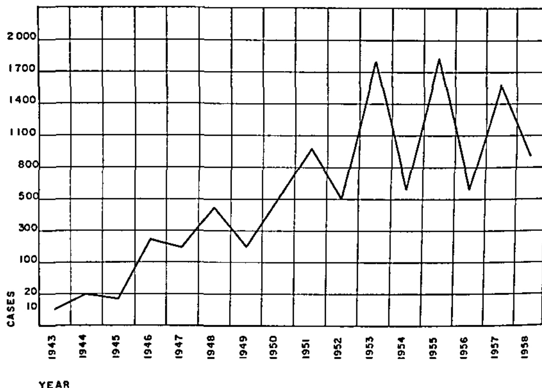


FIG 1 Poliomyelitis in the Republic of Mexico Number of reported paralytic cases from 1943 to 1958

February 1958, a small trial was carried out in Mexico City on 2,800 children mostly under 6 years of age. These children were living in 28 private nurseries distributed all over the city. They were brought to the institutions during the day until about 5 or 6 in the afternoon when parents or relatives picked them up to take them home for the night The viruses were fed separately at 3-week intervals in the recommended order Types 1, 3, and 2 From 17 February to 10 March 1958, 2,800 children were fed Type 1 virus in amounts of 0.1 ml. containing approximately $10^{5.[illegible]}$ TCD_{50}. From 11-31 March, 2,491 of these 2,800 children were fed Type 3 virus in amounts of $10^{5.[illegible]}$ TCD_{50} and from 1-21 April 2,090 of these children received Type 2 virus in amounts of $10^{5.[illegible]}$ TCD_{50}.

Clinical observations of these 2,800 children and of 9,000 contacts for a period of 8 months after the beginning of the trial, did not reveal any disease with CNS symptoms The minor illnesses such as diarrhea, mumps, measles, skin diseases, etc, observed in some of the children at various times during the observation period, could not be attributed to the administration of the vaccine It is important to mention that 1958 was a year of low incidence of paralytic poliomyelitis in Mexico, 123 cases having been reported in the whole year

Serological studies on paired sera obtained prior to the feeding of viruses 3 to 4 weeks after the last dose, in a randomly selected group, gave the following conversion rates: among 41 children negative for Type 1 virus, 74 per cent became positive after vaccination, out of 51 children negative for Type 2 virus, 70 per cent converted to positive; and among 36 negative for Type 3 virus 58 per cent converted to positive

The results of these preliminary studies, as well as the results reported by other investigators, gave confidence on the safety of the oral vaccine.

In August 1958 a decision was taken at the Ministry of Health of Mexico to start a large-

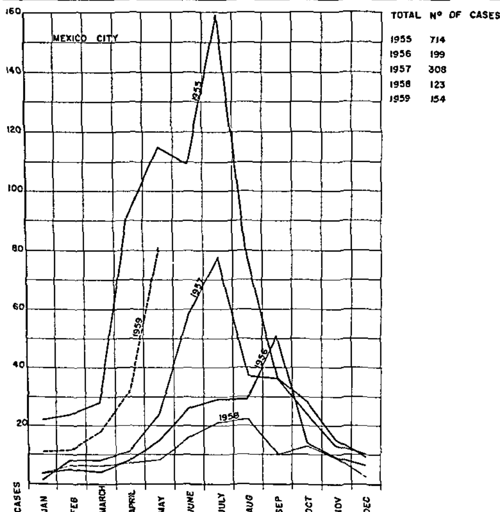


FIG 2 The seasonal distribution of poliomyelitis cases in Mexico City

scale vaccination program at the end of that year using Dr Sabin's live poliovirus vaccine Although steps were taken to carry out the vaccination during the months of October, November, and December 1958, this could not be accomplished until the end of February 1959 for reasons beyond our control This delay was most unfortunate because based on previous years' experience, we were expecting an epidemic in Mexico City in 1959, and this had indeed started early in January

The following should be mentioned in order to have an idea as to the kind of problem we were expecting

Of a small group of 12 children, from whom rectal swabs were obtained for special tests by Dr Sabin two weeks prior to the initiation of the large trial in Mexico City, 3 of them (25 per cent) were carriers of polioviruses Types 1 and 2, as Dr Sabin has already reported

This momentary sampling suggests how large a number of the children under 5 years of age

TABLE 2. POLIOMYELITIS ANTIBODY DISTRIBUTION IN CHILDREN OF THREE DIFFERENT CITIES BEFORE FEEDING SABIN'S ATTENUATED STRAINS—1959
(Children from 6 Months to 5 Years of Age)

City	Number Tested	Per Cent Positive for Indicated Type of Virus				
		None	Type 1	Type 2	Type 3	All 3
DISTRITO FEDERAL (Mexico City)	219	19	47	63	54	30
GUADALAJARA (Jalisco)	43	14	58	77	58	46
MONTERREY (Nuevo León)	47	17	66	42	66	36

were already infected with naturally-occurring polioviruses, and this age group is the most susceptible according to serological surveys conducted prior to feeding of viruses The serological tests to be presented were carried out in tube cultures of human kidney cells using the CPE method

Table 2 shows the antibody distribution for all 3 types of poliovirus prior to feeding of the vaccine in a randomly selected group of children in each of the 3 cities under study

Table 3 shows the data for Mexico City, broken down by individual age groups. As can be seen from this table, the incidence of neutralizing antibodies increased with age, by the time they reach the age of 3, the vast majority of the children have already acquired these antibodies These serological surveys are in line with clinical observations, the most susceptible children are under 2 years of age and it is precisely in this age group where the great majority of the paralytic cases are observed

TABLE 3 NEUTRALIZING ANTIBODIES FOR ALL 3 TYPES OF POLIOVIRUS IN THE SERA OF MEXICAN CHILDREN BEFORE FEEDING SABIN'S ATTENUATED STRAINS INCIDENCE ACCORDING TO AGE GROUPS
Mexico City 1959

Age Group Yrs	Number Tested	Per Cent Positive for Indicated Type of Virus				
		None	Type 1	Type 2	Type 3	All 3 Types
6—11 MONTHS	33	51	30	24	24	9
1—1 11	53	34	19	51	40	13
2—2 11	54	13	50	63	55	28
3—3 11	43	0	70	88	70	44
4—4 11	36	0	75	89	83	55
ALL	219	19	47	63	54	30

TABLE 4 ADMINISTRATION OF SABIN'S LIVE POLIOVIRUS VACCINE TO CHILDREN LIVING IN MEXICO CITY—1959

AGE GROUP YRS	NUMBER OF CHILDREN RECEIVING INDICATED TYPE OF VIRUS			
	TYPE 1 ONLY	TYPE 1 AND 3	TYPE 1 + 2 + 3	TOTAL
1	4,389	5,230	6,844	16,463
1—1 11	3,557	7,658	8,958	20,173
2—2 11	3,503	7,407	9,198	20,108
3—3 11	3,466	7,580	9,279	20,325
4—4 11	5,294	11,389	14,167	30,850
ALL	20,209	39,264	48,446	107,919

Table 4 shows the number of children in Mexico City who have received the various vaccine types of virus It is important to mention here, that Type 1 virus was fed from 23 February to 4 April, Type 3 virus from 5 April to 14 May, and Type 2 is being fed since 15 May

Table 5 shows the number of children who received Type 1 virus in the other 3 cities where the vaccine-feeding program began

It was in this setting, in which about 21 cases of paralytic poliomyelitis had already occurred, that we began feeding Type 1 vaccine in Mexico City on 23 February 1959 and during the subsequent 5 weeks administered it to 107,919 children from 6 months to 5 years of age (about 20 per cent of the total population of this age group in Mexico City) As stated, a large number of the vaccinated children were carrying other enteroviruses which interfered with the successful implantation of the Type 1 polio vaccine virus On these bases, the actual number of so-called "wild" polioviruses in the unvaccinated child

TABLE 5 ADMINISTRATION OF SABIN'S TYPE 1 ATTENUATED STRAIN TO MEXICAN CHILDREN IN 3 DIFFERENT CITIES—1959

AGE GROUP YRS	NUMBER OF CHILDREN FED IN INDICATED CITY			
	MONTERREY	GUADALAJARA	PUEBLA	ALL
6—11 MONTHS	4,884	2,966	845	8,695
1—1 11	7,310	4,131	1,266	12,707
2—2 11	6,699	4,183	1,239	12,121
3—3 11	6,506	4,272	1,331	12,109
4—4 11	7,222	6,384	2,252	15,858
ALL	32,621	21,936	6,933	61,490

TABLE 2 POLIOMYELITIS ANTIBODY DISTRIBUTION IN CHILDREN OF THREE DIFFERENT CITIES BEFORE FEEDING SABIN'S ATTENUATED STRAINS—1959
(Children from 6 Months to 5 Years of Age)

City	Number Tested	Per Cent Positive for Indicated Type of Virus				
		None	Type 1	Type 2	Type 3	All 3
DISTRITO FEDERAL (Mexico City)	219	19	47	63	54	30
GUADALAJARA (Jalisco)	43	14	58	77	58	46
MONTERREY (Nuevo León)	47	17	66	42	66	36

were already infected with naturally-occurring polioviruses, and this age group is the most susceptible according to serological surveys conducted prior to feeding of viruses The serological tests to be presented were carried out in tube cultures of human kidney cells using the CPE method

Table 2 shows the antibody distribution for all 3 types of poliovirus prior to feeding of the vaccine in a randomly selected group of children in each of the 3 cities under study

Table 3 shows the data for Mexico City, broken down by individual age groups. As can be seen from this table, the incidence of neutralizing antibodies increased with age, by the time they reach the age of 3, the vast majority of the children have already acquired these antibodies These serological surveys are in line with clinical observations; the most susceptible children are under 2 years of age and it is precisely in this age group where the great majority of the paralytic cases are observed

TABLE 3 NEUTRALIZING ANTIBODIES FOR ALL 3 TYPES OF POLIOVIRUS IN THE SERA OF MEXICAN CHILDREN BEFORE FEEDING SABIN'S ATTENUATED STRAINS INCIDENCE ACCORDING TO AGE GROUPS
Mexico City 1959

Age Group Yrs.	Number Tested	Per Cent Positive for Indicated Type of Virus				
		None	Type 1	Type 2	Type 3	All 3 Types
6—11 MONTHS	33	51	30	24	24	9
1—1.11	53	34	19	51	40	13
2—2 11	54	13	50	63	55	28
3—3 11	43	0	70	88	70	44
4—4 11	36	0	75	89	83	55
ALL	219	19	47	63	54	30

TABLE 8. ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 3 ANTIBODY WHO WERE FED SABIN'S TYPE 2 VACCINE
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		NUMBER IN INDICATED AGE GROUP FED TYPE 3 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 3 ANTIBODY		
6—11 months	33	76	12,074	9,176
1—1 11	53	60	16,616	9,969
2—2.11	54	45	16,605	7,472
3—3 11	43	30	16,859	5,057
4—4 11	36	17	25,556	4,344
Total	219	46	87,710	36,018

Table 9 shows the data for Type 1 virus in 3 of the 4 cities, the city of Puebla is not included because the serological surveys have not been completed. This table indicates that out of a total number of 160,456 children vaccinated with Type 1 virus, an estimated number of 72,020 did not have antibodies for this virus.

The epidemiological, as well as the clinical, observations have not been tabulated because the program is not finished, this is just a preliminary report We will only give the number of paralytic cases that have been observed in the 4 cities up to mid-June

Table 10 shows the data for Monterrey In this area, no cases of paralytic poliomyelitis have been reported since February There were only 3 cases reported in January.

In the city of Guadalajara, on the other hand (Table 11), it seemed that an epidemic was picking up from the beginning of the year The larger number of cases were reported earlier in May

TABLE 9 ESTIMATED NUMBER OF CHILDREN UNDER 5 YEARS OF AGE IN 3 DIFFERENT CITIES WITHOUT TYPE 1 ANTIBODY WHO WERE FED SABIN'S TYPE 1 VACCINE—1959

CITY	ESTIMATED POPULATION	NUMBER FED TYPE 1 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY
Mexico City	630,000	107,919	51,726
Monterrey	70,000	32,621	11,091
Guadalajara	66,428	21,936	9,203
All	766,428	160,456	72,020

population can be estimated to have greatly outnumbered those that we might have succeeded in implanting by the vaccine-feeding program

Four cities were selected for the vaccination program. Mexico City, located in the central part of the country; Puebla, approximately 100 miles south of Mexico City; Monterrey in the north, and Guadalajara in the west central part.

The viruses were fed separately at 4-to-5-week *intervals in amounts of 0 1 ml., representing* $10^{5.5}$ *to* $10^{5.9}$ TCD_{50}. *Type 1 virus was administered* first, followed by Type 3 and finally Type 2.

The vaccination program, initiated on 27 April 1959, was limited to children

Table 6 shows an estimated number of children without Type 1 antibody who were fed Type 1 virus in Mexico City. Out of 107,919 children fed Type 1 virus, an estimated number of 51,726 did not have antibodies for this type. Tables 7 *and 8 give similar data for Types 2 and 3 viruses*

TABLE 6 ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 1 ANTIBODY WHO WERE FED LIVE TYPE 1 POLIOVIRUS VACCINE—SABIN
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		NUMBER IN INDICATED AGE GROUP FED TYPE 1 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 1 ANTIBODY		
6—11 months	33	70	16,463	11,524
1—1 11	53	81	20,173	16,339
2—2 11	54	50	20,108	10,054
3—3 11	43	30	20,325	6,097
4—4 11	36	25	30,850	7,712
Total	219	53	107,919	51,726

TABLE 7 ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 2 ANTIBODY WHO WERE FED SABIN'S TYPE 2 VACCINE
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		*NUMBER IN INDICATED* AGE GROUP FED TYPE 2 VACCINE	*ESTIMATED NUMBER* WITHOUT TYPE 2 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 2 ANTIBODY		
6—11 months	33	76	6,844	5,201
1—1 11	53	49	8,958	4,389
2—2 11	54	37	9,198	3,403
3—3 11	43	12	9,279	1,113
4—4 11	36	11	14,167	1,558
Total	219	37	48,446	15,664

TABLE 8 ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 3 ANTIBODY WHO WERE FED SABIN'S TYPE 2 VACCINE
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		NUMBER IN INDICATED AGE GROUP FED TYPE 3 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 3 ANTIBODY		
6—11 months	33	76	12,074	9,176
1—1 11	53	60	16,616	9,969
2—2 11	54	45	16,605	7,472
3—3 11	43	30	16,859	5,057
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CITY	ESTIMATED POPULATION	NUMBER FED TYPE 1 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY
Mexico City	630,000	107,919	51,726
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All	766,428	160,456	72,020

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Four cities were selected for the vaccination program. Mexico City, located in the central part of the country; Puebla, approximately 100 miles south of Mexico City, Monterrey in the north, and Guadalajara in the west central part.

The viruses were fed separately at 4-to-5-week intervals in amounts of 0 1 ml, representing $10^{5.6}$ to $10^{5.9}$ TCD_{50}. Type 1 virus was administered first, followed by Type 3 and finally Type 2.

The vaccination program, initiated on 27 April 1959, was limited to children

Table 6 shows an estimated number of children without Type 1 antibody who were fed Type 1 virus in Mexico City. Out of 107,919 children fed Type 1 virus, an estimated number of 51,726 did not have antibodies for this type Tables 7 and 8 give similar data for Types 2 and 3 viruses

TABLE 6. ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 1 ANTIBODY WHO WERE FED LIVE TYPE 1 POLIOVIRUS VACCINE—SABIN
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		NUMBER IN INDICATED AGE GROUP FED TYPE 1 VIRUS	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 1 ANTIBODY		
6—11 months	33	70	16,463	11,524
1—1 11	53	81	20,173	16,339
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3—3 11	43	30	20,325	6,097
4—4 11	36	25	30,850	7,712
Total	219	53	107,919	51,726

TABLE 7 ESTIMATED NUMBER OF CHILDREN WITHOUT TYPE 2 ANTIBODY WHO WERE FED SABIN'S TYPE 2 VACCINE
Mexico City 1959

AGE GROUP YEARS	SEROLOGIC STUDIES		NUMBER IN INDICATED AGE GROUP FED TYPE 2 VACCINE	ESTIMATED NUMBER WITHOUT TYPE 2 ANTIBODY
	NUMBER TESTED	PERCENT NEGATIVE FOR TYPE 2 ANTIBODY		
6—11 months	33	76	6,844	5,201
1—1 11	53	49	8,958	4,389
2—2 11	54	37	9,198	3,403
3—3 11	43	12	9,279	1,113
4—4 11	36	11	14,167	1,558
Total	219	37	48,446	15,664

TABLE 12. CASES OF PARALYTIC POLIOMYELITIS IN THE CITY OF PUEBLA AFTER BEGINNING OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM—1959

MONTH	POPULATION ON 6 MONTHS TO 4 11 YRS OF AGE		TOTAL NO OF CASES OF PARALYTIC POLIOMYELITIS IN AGE GROUP 6 MONTHS—4 11 YRS		
	VACCINATED	UNVACCINATED	VACCINATED	UNVACCINATED	TOTAL
JANUARY			—	0	0
FEBRUARY			—	0	0
MARCH			—	0	0
APRIL	6,933	32,967	0	0	0
MAY			0	0	0
JUNE			0	2	2

TABLE 13 CASES OF PARALYTIC POLIOMYELITIS IN MEXICO CITY AFTER THE BEGINNING OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM—23 FEBRUARY 1959

MONTH	POPULATION IN AGE GROUP 6 MONTHS TO 5 YEARS OF AGE		TOTAL NO OF CASES OF PARALYTIC POLIOMYELITIS MOSTLY UNDER 4 11 YEARS OF AGE		
	VACCINATED	UNVACCINATED	VACCINATED	UNVACCINATED	TOTAL
JANUARY				10	10
FEBRUARY	107,919	422,081	0	11	11
MARCH			1	19	20
APRIL			2	39	41
MAY			10	61	71
JUNE			2	31	33
TOTAL	107,919	422,081	15	171	186

covered) have been observed among the vaccinated children, and 171 cases among non-vaccinated children

Only few of the viruses isolated from these cases have been typed Out of the 19 cases observed in March among the non-vaccinated children, virus isolations in 3 of them demonstrated Type 1 in one case and Type 2 in 2 cases Out of the 39 cases reported in April, among the non-vaccinated, studies of 4 of them showed that all 4 were due to Type 1 virus

The studies on the vaccinated children have not been completed However, 3 of them have been tested and their history is as follows

One child was a 1 year-7 months-old boy, who received Type 1 virus on 3 March He did not

TABLE 10 CASES OF POLIOMYELITIS IN THE CITY OF MONTERREY AFTER BEGINNING OF THE ORAL LIVE-POLIOVIRUS VACCINE PROGRAM—1959

MONTH	POPULATION ON 6 MONTHS TO 4 11 YRS OF AGE		TOTAL NO OF CASES OF PARALYTIC POLIOMYELITIS IN 6 MONTHS TO 4 11 YEARS OF AGE		
	VACCINATED	NON-VACCINATED	VACCINATED	UNVACCINATED	TOTAL
JANUARY				3	3
FEBRUARY				0	0
MARCH				0	0
APRIL	32,621	37,339	0	0	0
MAY			0	0	0

TABLE 11 CASES OF PARALYTIC POLIOMYELITIS *IN THE CITY OF GUADALAJARA AFTER THE BEGINNING* OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM—1959

MONTH	POPULATION ON 6 MONTHS TO 4 11 YRS OF AGE		TOTAL NO OF CASES OF PARALYTIC POLIOMYELITIS MOSTLY IN AGE GROUP 6 MONTHS TO 4 11—YRS		
	VACCINATED	NON-VACCINATED	VACCINATED	UNACCINATED	TOTAL
JANUARY			—	2	2
FEBRUARY			—	6	6
MARCH			—	1	1
APRIL	21,936	44,492	0	4	4
MAY			0	32	32

Only 2 cases were reported in June in the city of Puebla (*Table 12*).

An analysis of the data could not be made at the present time; however, it is interesting to note that not a single case of paralytic poliomyelitis occurred among 61,490 vaccinated children in 3 different cities.

Table 13 shows the data for Mexico City It should be emphasized here that from 23 February to 4 April 107,919 children were fed Type 1 virus, from 5 April to 14 May 87,710 received Type 3, and since 15 May 48,446 have received Type 2

This table shows the distribution of paralytic cases among the vaccinated as well as among the non-vaccinated children in different months It is important to mention that the epidemic we were expecting did occur; however, it should be emphasized that this epidemic began early in the year before the vaccination program was started. Thus far, as is shown in this table, 15 cases of paralysis (some of whom have completely re-

TABLE 12 CASES OF PARALYTIC POLIOMYELITIS IN THE CITY OF PUEBLA AFTER BEGINNING OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM—1959

Month	Population on 6 Months to 4 11 Yrs of Age		Total No of Cases of Paralytic Poliomyelitis in Age Group 6 Months—4 11 Yrs		
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Total
JANUARY			—	0	0
FEBRUARY			—	0	0
MARCH			—	0	0
APRIL	6,933	32,967	0	0	0
MAY			0	0	0
JUNE			0	2	2

TABLE 13 CASES OF PARALYTIC POLIOMYELITIS IN MEXICO CITY AFTER THE BEGINNING OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM—23 FEBRUARY 1959

Month	Population in Age Group 6 Months to 5 Years of Age		Total No of Cases of Paralytic Poliomyelitis Mostly Under 4 11 Years of Age		
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Total
JANUARY				10	10
FEBRUARY	107,919	422,081	0	11	11
MARCH			1	19	20
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TOTAL	107,919	422,081	15	171	186

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Only few of the viruses isolated from these cases have been typed Out of the 19 cases observed in March among the non vaccinated children, virus isolations in 3 of them demonstrated Type 1 in one case and Type 2 in 2 cases Out of the 39 cases reported in April, among the non vaccinated, studies of 4 of them showed that all 4 were due to Type 1 virus

The studies on the vaccinated children have not been completed However, 3 of them have been tested and their history is as follows

One child was a 1-year-7-months old boy, who received Type 1 virus on 3 March He did not

have any sign of disease during the following 5 weeks. On 15 April he received Type 3 virus and on 22 April he developed a fever, vomiting, and abdominal pain, a few days later he had a transitory paralysis of the left leg On 28 April, 4 days after onset of disease, Type 1 poliovirus was isolated from the stools A period of 47 days elapsed from the time he was fed Type 1 vaccine to the time he developed this transitory paralysis

The other child was a 1-year-6-months-old male who received Type 1 virus on 25 February and Type 3 on 8 April. On 5 May he had paralysis of the right leg Type 1 poliovirus was recovered from the stools on the first day of paralysis, which began 65 days after feeding of Type 1 virus.

The third case was a 1-year-old boy who received Type 1 virus on 12 April and Type 3 on 14 May On 25 May he had paralysis of the right leg A 40-day period elapsed between the time he was first vaccinated and the time of paralysis In this case, Type 1 poliovirus was isolated from the stools on the first day of paralysis.

A definite conclusion cannot be made on the basis of available data, however, we could very well consider these 3 cases as an example of failure of immunization. This failure of immunization should be expected in a number of vaccinated children. The data already reported leave little doubt concerning the important problems of interference of the vaccine virus with a number of different enteroviruses We know from previous studies carried out by Dr Sabin and Dr. Ramos Alvarez that a high percentage (at least 20 per cent) of children of the low socio-economic status in Mexico are infected at any given moment with one or another type of enterovirus, so that it is not very unlikely that an interference phenomenon, resulting in failure of immunization, may indeed have occurred in most of the vaccinated children in whom paralysis appeared

Although the serological studies for 1959 have not been completed, because the program is still under way, data of previous studies of children who were fed the vaccine in various doses and schedules are presented

Table 14 shows the antibody response of children fed 2 different doses of each of the 3 types of viruses at 2 or 3-week intervals It is interesting to see that the response of triple negative children to feeding of vaccine is somewhat similar to that observed in children who had one or two types of heterotypic antibody at the time of feeding.

The possibility of feeding all 3 types of virus simultaneously has been considered very often because of the practical importance of its applicability.

Table 15 shows the antibody response obtained in children fed all 3 types of virus at one time in comparison with the results obtained in children fed the viruses separately.

It is interesting to see from these studies, when all 3 viruses are fed together, that the response for Types 1 and 2 is better than when the viruses are fed separately This, however, was not the case for Type 3 virus

SUMMARY.—Clinical, epidemiological, and laboratory studies were carried out in 2,800 children fed Sabin's oral vaccine in Mexico in 1958 The clinical and epidemiological observations in these 2,800 children and in 9,000 contacts did not reveal any disease with CNS symptoms during an 8-month observation period after the beginning of the trial.

Serological studies in a randomly selected group of these 2,800 children gave the following conversion rates 9 weeks after feeding the first type and 6 weeks after feeding the second type for Type 1, 76 per cent, for Type 2, 20 per cent and Type 3, 58 per cent.

We attempt to draw no conclusions on the incomplete data available on the large trial still under way in 1959, except that it is evident that the phenomenon of interference is playing a part in the successful vaccination in at least a portion of the children

Table 14 Serologic Response of Children with Heterotypic Pre-Antibody for One or More Types and Children with no Demonstrable Pre-Antibody to Any Type to Feeding of Viruses in Two Different Doses Separately at 2 and 3 Week Intervals

Lacking Antibody	Children With Heterotypic Pre-Antibody For One or Two Types			Children With No Demonstrable Pre-Antibody For Any Type	
	Dose of Virus Fed TCD_{50}	Number in Group	Number Developing Lacking Antibody—Per Cent	Number in Group	Number Developing Antibody Per Cent
1	$10^{6.4}$	—	—	4	4—100%
	$10^{5.4}$	31	23— 74%	10	8— 80%
2	$10^{6.7}$	7	7—100%	4	4—100%
	$10^{5.7}$	41	29— 71%	10	7— 70%
3	$10^{6.9}$	9	8— 89%	4	2— 50%
	$10^{5.9}$	26	14— 54%	10	7— 70%

have any sign of disease during the following 5 weeks. On 15 April he received Type 3 virus and on 22 April he developed a fever, vomiting, and abdominal pain, a few days later he had a transitory paralysis of the left leg On 28 April, 4 days after onset of disease, Type 1 poliovirus was isolated from the stools A period of 47 days elapsed from the time he was fed Type 1 vaccine to the time he developed this transitory paralysis

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A definite conclusion cannot be made on the basis of available data; however, we could very well consider these 3 cases as an example of failure of immunization This failure of immunization should be expected in a number of vaccinated children The data already reported leave little doubt concerning the important problems of interference of the vaccine virus with a number of different enteroviruses We know from previous studies carried out by Dr Sabin and Dr. Ramos Alvarez that a high percentage (at least 20 per cent) of children of the low socio-economic status in Mexico are infected at any given moment with one or another type of enterovirus, so that it is not very unlikely that an interference phenomenon, resulting in failure of immunization, may indeed have occurred in most of the vaccinated children in whom paralysis appeared.

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SUMMARY.—Clinical, epidemiological, and laboratory studies were carried out in 2,800 childrend fed Sabin's oral vaccine in Mexico in 1958 The clinical and epidemiological observations in these 2,800 children and in 9,000 contacts did not reveal any disease with CNS symptoms during an 8 month observation period after the beginning of the trial

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We attempt to draw no conclusions on the incomplete data available on the large trial still under way in 1959, except that it is evident that *the phenomenon of interference is playing a part* in the successful vaccination in at least a portion of the children.

DISCUSSION

CHAIRMAN RHODES: The paper presented by Dr Ramos Alvarez is now open for discussion Dr Bodian

DR BODIAN: Both Dr Abad Gómez and Dr Ramos Alvarez have indicated that in their countries there has been an increasing incidence of paralytic polio over the last short period of years, and in both cases, I believe, the paralytic disease is largely confined to those below the age of two years

I wonder whether, in interpreting the increase, one should not consider the possibility of an increase in the accuracy of reporting It is a well known phenomenon that in many countries throughout the world over the last period of years an increase in the accuracy of reporting cases may account for at least a part of the apparent increase in paralytic polio This seems to me to be particularly likely in view of the fact that the disease is still confined to the infantile group, whereas if it were the type of increase which we had been experiencing in the past quarter century in the United States and in Europe, and elsewhere throughout the world, it would be coincident with a change in age selection

Therefore, I believe that before we can assume that we are dealing with an increase in the actual incidence, we ought to consider the role of reporting

There are several other minor comments I should like to make.

First of all, I think that each of these studies we have heard this morning are extremely important and will contribute to progress in this field I was impressed with Dr. Abad Gómez' report, in that it seems to me that with careful surveillance in the future and with the application of wisdom, and perhaps putting faith aside for a while, we may learn from the Andes experience what the persistence of immunity may be, and other things, but certainly at the present time we must keep in abeyance any interpretation of that study

In relation to the Nicaraguan experience, one of the most impressive things to me was the fact that here we had a bona fide Type 2 epidemic, and this epidemic was completed at the time of the onset of immunization.

Now, I was wondering, since we know so very little about Type 2 epidemics and their effect upon subsequent heterotypic immunity, whether we should not hold in abeyance an interpretation of the freedom from paralytic polio in the eight months after the program

It is possible that the occurrence of a Type 2 epidemic of such scope may be playing a role, and I think it will be very interesting to see what happens in the coming summer and in the following periods of epidemic prevalence

One additional point I would like to bring out in relation to the Nicaraguan study is that I was not entirely clear about the intensity of vaccination with formalinized vaccine in the "unvaccinated group" The report does indicate the degree in the "vaccinated group," and I would like to have Dr Berrios comment on that

Conceivably, there might be a slight influence there as well

CHAIRMAN RHODES Dr López Berrios, will you comment?

DR LÓPEZ BERRIOS *(through an interpreter)* Of the children who were fed the three strains of the vaccine, 6 4 per cent had received one injection of Salk vaccine in 1958, 2 3 per cent had received two shots, and 0 9 per cent had received three shots

DR BODIAN: I knew of that data, Dr Berrios, which is given in your report I asked about the rate of formalinized vaccine administration in those who did not receive the live virus vaccine

CHAIRMAN RHODES Can you answer that, Dr. López Berrios?

DR LÓPEZ BERRIOS *(through an interpreter)* No We only took into account the percentage among the orally-vaccinated children However, we did vaccinate with the three viruses 82 per

Table 15. Summary of Tests Concerning the Serologic Response in Children Lacking Antibody for Any One Type After Feeding Sabin's Attenuated Strains Separately at 2 or 3 Week Intervals or Simultaneously

Lacking Antibody	Dose of Virus Fed TCD_{50}	Children Fed Viruses Separately		Children Fed Viruses Simultaneously	
		Number Tested	Per Cent Developing Lacking Antibody	Number Tested	Per Cent Developing Lacking Antibody
1	$10^{6.8}$	4	100	26	92
	$10^{4.6}$	41	76	23	82
2	$10^{6.7}$	11	100	29	93
	$10^{4.7}$	51	70	26	88
3	$10^{6.9}$	13	77	11	46
	$10^{5.9}$	36	58	23	39

11. REPORT ON FIELD TRIALS WITH LIVE ATTENUATED POLIOMYELITIS VACCINE IN POLAND

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R. STANCZYK AND D. NARUSZEWICZ

The Virology Department of the State Institute of Hygiene
Warsaw, Poland

DR PRZESMYCKI *(presenting the paper)*: As a result of suggestions at a conference of the Expert Committee on Poliomyelitis held in Copenhagen in 1958,[1] we initiated the following studies with live attenuated vaccine

(1) Clinical observation of persons vaccinated with live attenuated vaccine

(2) Propagation of virus in the intestinal tract of vaccinated persons

(3) Spread of the virus among contacts of vaccinated persons

(4) Immunizing properties of the vaccine

(5) Stability of strains following their passage through human intestines

Dr H Koprowski supplied us with the CHAT strain of Type 1 live attenuated poliomyelitis vaccine The vaccine was frozen and kept at -20°C until it was used.

Preliminary Investigation in Our Laboratory

The infectivity titer for monkey kidney tissue culture was $10^{5.25}$ $TCID_{50}$ per ml

Four *Macaca rhesus* monkeys and 3 *Macaca cynomolgus* monkeys were inoculated intracerebrally with undiluted vaccine, 1 ml per monkey Twelve *Macaca rhesus* and 12 *Macaca cynomolgus* monkeys were inoculated intraspinally. Twelve of the monkeys inoculated intraspinally were given 0.1 ml. of undiluted vaccine, and 12 were given the same quantity of vaccine diluted to 10^{-1}. The monkeys were under clinical observation for 18 days They were then sacrificed and their brains and spinal cords were examined histologically The results of these studies are presented in Table 1.

In the intracerebrally inoculated monkeys, no clinical symptoms were observed, and no lesions could be found upon histological examination Among the intraspinally inoculated animals, the *Macaca rhesus* monkeys showed no clinical symptoms or histologic changes In the *Macaca cynomolgus* monkeys, however, there were 2 cases of slight paralysis and, in addition, 2 other cases in which histological changes were observed The changes in the central nervous system which appeared in the 4 monkeys are within the limits generally accepted in tests of attenuated live vaccine. It should be added that in the

TABLE 1 PATHOGENICITY FOR MONKEYS OF CHAT VIRUS PREPARATION* (LOT #13) USED FOR ORAL IMMUNIZATION OF MAN

SPECIES	SIGNS	RATIO OF POLIO-INFECTED MONKEYS		
		INTRACEREBRAL	INTRASPINAL	
		UNDILUTED	UNDILUTED	1 10
Rhesus	Clinical	0/4	0/6	0/6
	Histologic	0/4	0/6	0/6
Cynomolgus	Clinical	0/3	1/6	1/6
	Histologic	0/3	2/6	2/6

* $10^{7.25}$ $TCID_{50}/ml$

cent of the children under 10 years of age in Managua

Chairman Rhodes: Dr. Barr.

Dr. Barr: I am surprised that no one in this group has picked up the statement that Dr Ramos Alvarez made to the effect that he had a very marked decrease in infant mortality as a result of this study. It seems to me that it is just as important to get all of the fringe benefits, if you want to call them that, out of these programs as it is to get the information for which the study was designed. And if these are of value, certainly we have added that much more to our program

Dr. Ramos Alvarez mentioned, as will be recalled, that he had mortality rates from 1954 to 1959 of 89, 21, 15, 39, 22, and then in 1959 the rate dropped to 15 This happened in other states in Mexico as well as in Colombia

Chairman Rhodes: That is a lengthy question to answer. Is there a short answer to it?

Dr Ramos Alvarez: No.

Chairman Rhodes No from Mexico; is there any other answer?

I believe that it would probably be better to discuss this later, the discussion could become involved. I think that short answers to vital statistics are not to be recommended, anyway.

TABLE 3 AGE DISTRIBUTION OF VACCINEES IN WYSZKOW AND ITS NEIGHBORING DISTRICT

AGE (YEARS)	NUMBER OF VACCINATED
½—3	551
4—6	549
7—10	811
11—15	709
16—18	242
Age not determined	26
Total	2,888

A boarding school for boys was studied separately as a group under special observation. At the time there were 70 boys at this school. Fifty per cent of these boys were vaccinated, the other 50 per cent were given only milk without vaccine. The study was arranged so that half the boys in each dormitory were vaccinated, while the other half received only milk.

Feces for virologic studies and blood for serologic studies were collected from all the boys under observation. Feces were collected prior to vaccination and 24 hours, 5 days, 10 days, 20 days, 30 days, and 90 days after vaccination.

Results of Virus Excretion Studies

Data on virus excretion by boys of the Wyszkow boarding school are presented in Table 4. The virus was isolated from the feces of 23 of the 32 boys who had been vaccinated (72 per cent). In view of the relatively small number of boys who were examined, it is difficult to ascertain the correlation between the level of antibodies and the virus excretion. Among the boys who were not vaccinated but were living in close contact with those who were vaccinated, the virus was isolated in 5 out of 30 persons (17 per cent). The highest percentage of positive isolations in vaccinated persons was found within 20 days. In 3 cases the virus was isolated after 90 days. The titer of excreted virus ranged from 10^2 to 10^5 $TCID_{50}$ per gram of feces.

TABLE 4 EXCRETION OF CHAT VIRUS BY INDIVIDUALS FED VIRUS AND BY THEIR CONTACTS
Wyszkow Boys' School

ANTIBODY LEVEL BEFORE VACCINATION	RATIO OF POSITIVE VIRUS EXCRETION	
	FED VIRUS	CONTACTS
<1 4	8/9	2/5
1 4—1 64	1/3	1/7
>1 64	8/12	2/15
Not determined	6/8	0/3
Total	23/32 (72%)	5/30 (17%)

TABLE 5 EXCRETION OF CHAT VIRUS BY CHILDREN FED VIRUS AND BY THEIR FAMILY CONTACTS
Warsaw families

ANTIBODY LEVEL BEFORE VACCINATION	RATIO OF POSITIVE VIRUS EXCRETION	
	FED VIRUS	CONTACTS
<1 4	3/3	2/4
1 4—1 64	9/9	3/8
>1 64	5/6	1/9
Not determined	2/4	1/3
Total	19/22 (86%)	7/24 (29%)

protocols sent by Dr Koprowski, the data showed that this vaccine induced clinical pathological symptoms in only 2 *Macaca rhesus* monkeys of the 35 which had been inoculated.

The laboratory tests with the vaccine were followed by 2 field trials one in the provincial town of Wyszkow and its neighboring villages, the other in Warsaw.

Materials and Methods

Feces. Feces were collected and kept frozen at −20°C. until used After thawing, a suspension of the feces in Hanks' solution was prepared This suspension was shaken for 30 minutes in a bottle with glass beads, treated by freezing and thawing two times, and centrifuged at 2,000 r p m for 30 minutes The supernatant fluid was centrifuged once more at 3,000 r p m. for 30 minutes Sodium bicarbonate solution was added to the fluid to bring it to a pH of 7 6 The following antibiotics were added to the suspension penicillin 100 units per ml. and streptomycin 100 units per ml Each sample of feces was used to infect 4 tubes of monkey kidney tissue culture After 7 days the results were read The positive samples were identified in a neutralization test with standard reference sera

Blood Blood was collected aseptically before vaccination, and 2 months following vaccination The separated serum was inactivated at 56°C for 30 minutes and kept at −20°C until the time of the test The sera were studied for neutralization of the cytopathogenic effect of poliovirus in tissue culture The serum under investigation was diluted in logarithmic series Each dilution of the standard virus was diluted so as to contain 100 $TCID_{50}$ The mixture of virus and serum was kept at 37°C. for 6 hours, then 2 tubes of kidney tissue culture were inoculated with one dose of serum. Seven days later, the results were read

Intraspinal Inoculation in Monkeys

The inoculum was injected into the intervertebral space in the lumbar portion of the spine between the 4th and 5th vertebrae The needle was inserted into this part of the spinal cord, and only after inducing a pronounced reflex of the lower extremity was the vaccine under examination injected. The inoculated monkeys were under observation for 18 days; each day they were examined clinically for neurological symptoms (temperature, reflexes, paresis, flaccid paralysis). After this observation period, the monkeys were sacrificed and sections of brain and spinal cord were collected for histopathological research

Research in Wyszkow and its Neighborhood

The research was conducted in the small, provincial town of Wyszkow and its neighboring villages The sanitary conditions of the town, which had been destroyed during the war, are poor, it lacks both a central water supply and a sewage system. During the past two years, no cases of poliomyelitis had been reported

A list of the population between 6 months and 16 years of age was prepared and each individual on the list was notified to appear for vaccination The vaccine was diluted 1 500 and served in milk. Each individual received about 200,000 $TCID_{50}$ doses The vaccine was kept in cold storage during the entire vaccination period The samples of the vaccine were taken immediately after the diluting process, and as soon as the vaccination was over the samples were tested in tissue culture These studies showed that the diluted vaccine retained the initial concentration of the virus until the end of vaccination. The vaccination program was so organized that every person was given the vaccine, already prepared for oral administration, and then samples of blood were collected

A total of 2,888 people, or 95 per cent of all those between the ages of 6 months and 16 years, were vaccinated (Table 2). The age distribution of vaccines is given in Table 3

TABLE 2 WYSZKOW TRIAL—PROPORTION OF POPULATION SUMMONED TO BE FED CHAT VIRUS

LOCALITY	POPULATION	NUMBER SUMMONED TO BE VACCINATED	NUMBER RESPONDING
Town of Wyszkow	5,714	2,050	1,970
Neighboring villages	3,004	963	918
Total	8,718	3,013	2,888

TABLE 3 AGE DISTRIBUTION OF VACCINEES IN WYSZKOW AND ITS NEIGHBORING DISTRICT

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4—6	549
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Age not determined	26
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A boarding school for boys was studied separately as a group under special observation At the time there were 70 boys at this school. Fifty per cent of these boys were vaccinated, the other 50 per cent were given only milk without vaccine The study was arranged so that half the boys in each dormitory were vaccinated while the other half received only milk

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Results of Virus Excretion Studies

Data on virus excretion by boys of the Wyszkow boarding school are presented in Table 4. The virus was isolated from the feces of 23 of the 32 boys who had been vaccinated (72 per cent). In view of the relatively small number of boys who were examined, it is difficult to ascertain the correlation between the level of antibodies and the virus excretion. Among the boys who were *not vaccinated but were living in close contact* with those who were vaccinated, the virus was isolated in 5 out of 30 persons (17 per cent). The highest percentage of positive isolations in vaccinated persons was found within 20 days In 3 cases the virus was isolated after 90 days. The titer of excreted virus ranged from 10^{3} to 10^{5} TCID$_{50}$ per gram of feces

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Wyszkow Boys' School

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	FED VIRUS	CONTACTS
<1 4	8/9	2/5
1 4—1 64	1/3	1/7
>1 64	8/12	2/15
Not determined	6/8	0/3
Total	23/32 (72%)	5/30 (17%)

TABLE 5 EXCRETION OF CHAT VIRUS BY CHILDREN FED VIRUS AND BY THEIR FAMILY CONTACTS
Warsaw families

ANTIBODY LEVEL BEFORE VACCINATION	RATIO OF POSITIVE VIRUS EXCRETION	
	FED VIRUS	CONTACTS
<1 4	3/3	2/4
1 4—1 64	9/9	3/8
>1 64	5/6	1/9
Not determined	2/4	1/3
Total	19/22 (86%)	7/24 (29%)

Vaccination in Warsaw

In Warsaw, children from 12 families were vaccinated and kept under rigid observation. The same dose of vaccine was used as in Wyszkow, i.e. 200,000 $TCID_{50}$. Some of the children had already been vaccinated 2 or even 3 times with Salk's inactivated vaccine. The children and their relatives were examined for the presence of virus in feces before vaccination. The level of neutralizing antibodies in their blood was also examined. The vaccine was administered in milk as was done in Wyszkow. Feces, both of the children and other members of their families, were studied at the same time as in Wyszkow. The results are presented in Table 5.

Poliovirus was isolated from the feces of 86 per cent of vaccinated children.

The percentage of vaccinated children excreting virus was higher in Warsaw than in the Wyszkow Boarding School. The results may have been affected by the age of the vaccinated, on the whole, children vaccinated in Warsaw were younger than the boys in the Wyszkow Boarding School.

A higher percentage of isolation among the relatives of the vaccinated children in Warsaw, as compared with contacts in the Wyszkow Boarding School, may be explained by the closer contact of vaccinated children with members of their families and by the greater percentage of children excreting virus.

Effect of the presence of cytopathogenic agents other than poliovirus upon the course of immunization

Stools of 70 boarding school boys who had not been previously vaccinated were collected 3-4 days *before* administration of CHAT virus. Attempts to isolate virus cytopathogenic for monkey kidney tissue culture system yielded positive results in 6 cases. None of the 6 isolates was polio virus. Attempts to identify the agents have not yet been completed. Preliminary results of immunization of these 6 children with CHAT virus as well as 2 contacts who excreted virus are summarized in Table 6. It may be observed that in at least 5 of 6 cases the unknown agent failed to interfere with the course of intestinal infection induced by ingestion of CHAT virus. Case #39 merits particular attention. The cytopathogenic agent was present together with poliovirus on the 5th and 20th day after feeding CHAT virus and it is quite probable that it was present concurrently during the entire time of observation. This child had a 1:512 titer of Type 1 antibody before feeding, caused obviously by past natural infection. Normally, one might have expected a resistance to intestinal infection with Type 1 virus and yet the opposite was found, as indicated by the duration of intestinal infection and rise in homotypic antibody titer. It may be interesting also to point out that only in this one case (out of 11 tested) was the excreted virus slightly pathogenic for monkeys, as shown subsequently in Table 11. Data related to the remaining three cases where the presence of a cytopathogenic agent was observed have not as yet been analyzed completely. However, it is quite certain that the presence of a cytopathogenic agent other than polio at the time of feeding of poliovirus does not always lead to interference. The agent isolated in these 6 cases may have been responsible for an epidemic of intestinal infection clinically characterized by diarrhea and vomiting which occurred a few weeks before administration of the CHAT strain. Work on identification of the agent is being continued.

Immunizing Effect of the Vaccine

In order to study the immunizing properties of the vaccine, 697 persons were randomly selected for bleeding before vaccination. The results are presented in Table 7. One hundred fourteen persons in whom no antibodies against Type 1 poliomyelitis virus were found, were re-bled 2 months after vaccination in order to determine the immunizing effect of the vaccine.

Table 8 indicates that no antibodies were found at a 1:4 dilution of serum in 10 per cent of the individuals after vaccination. Antibodies were found in quantities ranging from 1:4 to 1:32 in 26 per cent of those vaccinated, in 35 per cent of the cases, antibodies were found in titers ranging from 1:64 to 1:256, and in 29 per cent antibodies were found in dilutions of 1:512 and higher. Thus titers of 1:64 and 1:512 and higher were found in over 50 per cent of the group.

Eighty-four persons who had antibodies against Type 1 poliomyelitis virus before vaccination, were examined serologically. The results are

TABLE 6 INSTANCES IN WHICH NON-POLIO ENTERIC VIRUSES WERE ISOLATED FROM THE STOOL BEFORE VACCINATION

Case No	Strains Isolated Before Vaccination	Strains Isolated After Vaccination							Antibodies Type 1	
		24 Hrs	5 Days	10 Days	20 Days	30 Days	90 Days	150 Days	Before Vaccination	After Vaccination
39 vaccinated	UCA*	UCA	Polio Type 1	Neg	Polio Type 1	UCA	Neg	Neg	512	>1024
67 vaccinated	UCA	Neg.	Polio Type 1	Neg	Polio Type 1	Neg	Polio Type 1	Neg	Not tested	Not tested
52 vaccinated	UCA	Neg.	Polio Type 1	Polio Type 1	Neg	Neg	Neg.	Neg	64	2048
53 vaccinated	UCA	Neg	Neg	Neg	Neg	Neg.	Not tested	Neg	16	512
22 contact	UCA	Polio Type 1	Neg	Neg	Not tested	Not tested	Neg	Neg	512	512
54 contact	UCA	Neg	Neg	Not tested	Neg	Polio Type 1	Neg	Neg	16	512

* UCA—Unidentified cytopathogenic agent, possibly a member of the ECHO group.

TABLE 7 SEROLOGICAL SURVEY—WYSZKOW AND NEIGHBORING VILLAGES

Age Years	Total Tested	Triple Negative %	Negative Only for Type 1 %	Antibodies Type 1 %	Antibodies Type 2 %	Antibodies Type 3 %
½—3	94	22 4	32 9	44 7	57 4	58 5
4—5	156	11 5	18 6	69 9	60 2	60 9
7—10	162	4 4	14 8	80 8	75 5	76 5
11—15	204	2 9	15 2	81 9	79 5	82 3
16—20	81	3 7	7 4	88 9	87 6	86 4
Total	697					

TABLE 8 ANTIBODY RESPONSE FOLLOWING FEEDING OF CHAT VIRUS TO TYPE 1 NEGATIVE CHILDREN (WYSZKOW TRIAL)

Antibodies Against Virus Types—Before Vaccination (types)	Number of Subjects	Number of Subjects With Type 1 Antibodies at Various Levels, After Vaccination			
		<4	4-32	64-256	512 or more
None	35	4	11	14	6
2	17	2	4	6	5
3	20	2	5	4	9
2 & 3	42	3	10	16	13
Total	114	11 (10%)	30 (26%)	40 (35%)	33 (29%)

presented in Table 9, which shows that in 29 per cent of the individuals there was less than a four-fold increase in antibodies However, it should be emphasized that the individuals of this group had a rather high level of pre-existing antibodies, ranging from 1 32 to 1 256 Four-fold or higher antibody rises were found in 71 per cent of the examined individuals

The antibodies in the sera of 26 boys from the Wyszkow Boarding School were also examined before and after vaccination. All 8 individuals in the vaccinated group who had no antibody against

TABLE 9 HOMOTYPIC ANTIBODY RISE IN WYSZKOW CHILDREN WHO HAD TYPE 1 ANTIBODIES BEFORE CHAT FEEDING

	Rise in Antibodies					Total
	None	2X	4X	8X	>8X	
Number of Subjects	12	13	14	15	30	84
Per cent of Subjects	14	15	17	18	36	100

TABLE 10 INCREASE OF ANTIBODIES AFTER VACCINATION—BOARDING SCHOOL FOR BOYS

Titer	Prior to Vaccination*	Following Vaccination
2,048		oooooooo
1,024		ooooo
512	o	oooo
256	oooooo	o
128	oooo	ooo
64	ooo	ooo
32	o	o
16	oo	
8		
4	o	
<4	oooooooo	

* Each circle represents one person

poliomyelitis virus before inoculation, had Type 1 antibodies afterwards, and persons with pre-existing antibodies had increased titers of those antibodies ranging from 1:64 to 1:2,048 (Data obtained as shown in Table 10)

Neuropathogenic properties of excreted polio virus

Eleven strains isolated from feces collected from vaccinated persons and from those in contact with them were examined The strains used were isolated after a relatively long interval following feeding (20 or 30 days in most instances), on the assumption that virus, after a long period of multiplication is man's intestines, might acquire some neuropathogenic properties for monkeys Strains isolated from persons living in contact with the vaccinated were considered as strains that had had two passages in man A virus suspension isolated after one passage through monkey kidney tissue culture was used for inoculating the *Macaca rhesus* monkeys Each isolate was tested on 6 monkeys, 2 of which were inoculated intracerebrally with 1 ml of undiluted virus suspension, 2 with 0 1 ml of undiluted virus suspension intraspinally, and 2 with 0 1 ml of virus suspension, diluted to 10^{-1}, intraspinally

Results were evaluated on the basis of both clinical observations and histopathologic examination Data relating to pathogenicity are shown in Table 11 for virus from the stools of vaccinated children, and in Table 12 for virus from the stools of persons who had been in contact with vaccinated children.

Virus excreted by 5 of the 6 vaccinated children produced no clinical symptoms or histologic lesions in monkeys, whether injected intracerebrally or intraspinally. (See Table 11, #25, #42, #33, #1, #14) In the one exception (#39) the virus when injected intracerebrally caused a histologic lesion in one of the two monkeys, although neither of the monkeys showed clinical symptoms Virus from this same subject when inoculated intraspinally, caused histologic changes in all of 4 monkeys, inducing paralysis in two. However, an unidentified cytopathogenic agent was isolated from the stools of this child both before and after poliomyelitis infection

Virus excreted by persons who had been in contact with vaccinated children in most cases produced no clinical symptoms or histologic lesions in intracerebrally or intraspinally inoculated monkeys (See Table 12, #2, #43 after 10 days, #8) Virus from #35 produced no clinical symptoms or histologic lesions when inoculated intracerebrally When inoculated intraspinally, it caused paralysis in one of four monkeys The spinal cord of this monkey showed lesions of poliomyelitis Virus excreted by #43

TABLE 11. PATHOGENICITY FOR MONKEYS OF CHAT VIRUS ISOLATED FROM CHILDREN FED VIRUS

SUBJECT No	DAYS AFTER FEEDING	$TCID_{50}$† PER 0 1 ML	RATIO OF POLIO-INFECTED MONKEYS		
			INTRACEREBRAL	INTRASPINAL	
			UNDILUTED	UNDILUTED	1 10
39*	20*	7 00	1/2*	2/2*	2/2*
25	20	7 75	0/2	0/2	0/2
42	20	6 85	0/2	0/2	0/2
33	30	6 55	0/2	0/2	0/2
1	30	6 85	0/2	0/2	0/2
14	90	6 45	0/2	0/2	0/2

* Unidentified cytopathogenic agent isolated from subject before and after polio infection
† After one passage in monkey kidney tissue culture

TABLE 8. ANTIBODY RESPONSE FOLLOWING FEEDING OF CHAT VIRUS TO TYPE I NEGATIVE CHILDREN (WYSZKOW TRIAL)

ANTIBODIES AGAINST VIRUS TYPES—BEFORE VACCINATION (TYPES)	NUMBER OF SUBJECTS	NUMBER OF SUBJECTS WITH TYPE ANTIBODIES AT VARIOUS LEVELS AFTER VACCINATION		
		<4	4-32	64-256
None	35	4	11	14
2	17	2	4	6
3	20	2	5	4
2 & 3	42	3	10	16
Total	114	11 (10%)	30 (26%)	40 (3

presented in Table 9, which shows that in 29 per cent of the individuals there was less than a four-fold increase in antibodies However, it should be emphasized that the individuals of this group had a rather high level of pre-existing antibodies, ranging from 1.32 to 1.256. Four-fold or higher antibody rises were found examined individuals.

The antibodies in the Wyszkow Boarding Sch before and after vaccina the vaccinated group w

TABLE 9 HOMOTYPIC ANTIBODY RISE IN WYSZKOW CHILDREN ANTIBODIES BEFORE CHAT FEEDING

	RISE IN ANTIBODIES			
	NONE	2X	4X	8X
Number of Subjects	12	13	14	15
Per cent of Subjects	14	15	17	18

TABLE 10 INCREASE OF ANTIBODIES AFTER VACCINATION—BOARD

TITER	PRIOR TO VACCINATION*	FOLL
2,048		
1,024		o
512	o	ooo
256	oooooo	o
128	oooo	ooo
64	ooo	ooo
32	o	o
16	oo	
8		
4	o	
<4	oooooooo	

* Each circle represents one person

poliomyelitis virus before inoculation, had Type 1 antibodies afterwards, and persons with pre-existing antibodies had increased titers of those antibodies ranging from 1:64 to 1:2,048 (Data obtained as shown in Table 10.)

Neuropathogenic properties of excreted polio virus

Eleven strains isolated from feces collected from vaccinated persons and from those in contact with them were examined. The strains used were isolated after a relatively long interval following feeding (20 or 30 days in most instances), on the assumption that virus, after a long period of multiplication in man's intestines, might acquire some neuropathogenic properties for monkeys. Strains isolated from persons living in contact with the vaccinated were considered as strains that had had two passages in man. A virus suspension isolated after one passage through monkey kidney tissue culture was used for inoculating the *Macaca rhesus* monkeys. Each isolate was tested on 6 monkeys, 2 of which were inoculated intracerebrally with 1 ml. of undiluted virus suspension, 2 with 0.1 ml of undiluted virus suspension intraspinally, and 2 with 0.1 ml of virus suspension, diluted to 10^{-1}, intraspinally.

Results were evaluated on the basis of both clinical observations and histopathologic examination. Data relating to pathogenicity are shown in Table 11 for virus from the stools of vaccinated children, and in Table 12 for virus from the stools of persons who had been in contact with vaccinated children.

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TABLE 11. PATHOGENICITY FOR MONKEYS OF CHAT VIRUS ISOLATED FROM CHILDREN FED VIRUS

SUBJECT No	DAYS AFTER FEEDING	$TCID_{50}$† PER 0.1 ML	RATIO OF POLIO-INFECTED MONKEYS		
			INTRACEREBRAL	INTRASPINAL	
			UNDILUTED	UNDILUTED	1:10
39*	20*	7.00	1/2*	2/2*	2/2*
25	20	7.75	0/2	0/2	0/2
42	20	6.85	0/2	0/2	0/2
33	30	6.55	0/2	0/2	0/2
1	30	6.85	0/2	0/2	0/2
14	90	6.45	0/2	0/2	0/2

* Unidentified cytopathogenic agent isolated from subject before and after polio infection
† After one passage in monkey kidney tissue culture

TABLE 12 PATHOGENICITY FOR MONKEYS OF CHAT VIRUS ISOLATED FROM CONTACTS. TESTS DONE AFTER ONE PASSAGE IN TISSUE CULTURE

Subject No	Days After Contact	$TCID_{50}$ Per 0 1 Ml	Ratio of Polio-Infected Monkeys		
			Intracerebral	Intraspinal	
			Undiluted	Undiluted	1:10
35	5	6 75	0/2	0/2	1/2
2	10	7 15	0/2	0/2	0/2
43	10	7 00	0/2	0/2	0/2
8	20	6 75	0/2	0/2	0/2
43	50	6 95	0/2	0/2	1/2

after 50 days induced no clinical symptoms when inoculated intracerebrally; when an inoculum diluted to 10^{-1} was injected intraspinally, 1 monkey out of 4 became paralyzed after 24 hours, but that the effect was due to poliomyelitis infection was questionable as judged by histologic examination Thus, of a total of 22 monkeys inoculated intracerebrally, none showed any clinical symptoms and only one showed histologic polio lesions.

Of the 44 monkeys inoculated intraspinally, 4 showed paralytic symptoms Histologic examination showed that in one monkey these symptoms were questionable and we therefore consider this paralysis as traumatic Histological examination indicated that 5 of those 44 monkeys showed changes typical for polio

Spread of Type 1 virus in non vaccinated adult population of Wyszkow

For several weeks before vaccination of children in Wyszkow, stool specimens were collected from 398 individuals and tested for the presence of polio virus through inoculation of HeLa cell tissue cultures. Two weeks after vaccination was completed, stools were again collected from the members of families that had been in direct contact with a vaccinated child, and from persons who had had no known contact with those receiving virus Stools were again collected from these two groups 3 months later. (Results of this study are shown in Table 13.) Type 1 polio virus was isolated from the stools of 5 subjects out of 398 specimens tested Three of the excretors were siblings. The same number of subjects was found to excrete Type 2 virus and again three siblings were involved Seven subjects excreted cytopathogenic viruses not neutralized by any of the polio-immune sera This low incidence of spread of polio Type 1 virus in the Wyszkow community was maintained throughout the 3 months observation period following vaccination with the attenuated strain No differences were observed between the group of subjects in immediate contact with the immunized children and those who were supposed to have no contact at all

Discussion

In planning these experiments with the CHAT strain, we tried to create conditions as similar as possible to those under which we would eventually have to carry out mass vaccinations

Plotkin, Koprowski, Richardson, and Stokes,[3] in their field trials with families in Moorestown, showed that the CHAT strain of attenuated polio virus was isolated in 100 per cent of vaccinated children (18/18) In unvaccinated children who were in contact with the vaccinated ones, virus was isolated in 37.4 per cent (13/35), and in parents living in contact with their vaccinated children, virus was isolated in 8 5 per cent (3/36).

Buser and Schär's studies with the same strain[2] showed that virus was isolated in 100 per cent of newborn babies and children who had no antibodies (1:5) before vaccination; in children with antibodies (titer 1:5 to 1:50), virus was isolated in 86 per cent, and in those with

Table 13 * Studies of Spread of Poliomyelitis Virus in Non-Vaccinated Population of Town of Wyszkow Before and After Vaccination of Children with CHAT Virus

Group	Stool Collection	Ratio Excreting Virus: Families Type 1	Families Type 2	Families Not Polio	Individuals Type 1	Individuals Type 2	Individuals Not Polio
All Contacts	Before vac	3/128	3/128	7/128	5/398	5/398	7/398
		1/36	0/36	0/36	1/68	0/68	0/68
	2 weeks after vaccination						
No Contact Contacts		0/55	1/55	2/55	0/134	1/134	2/134
		0/30	0/30	0/30	0/107	0/107	0/107
	3 months after vaccination						
No Contact		0/53	0/53	1/53	0/122	0/122	1/122

* After Georgiades

TABLE 12. PATHOGENICITY FOR MONKEYS OF CHAT VIRUS ISOLATED FROM CONTACTS TESTS DONE AFTER ONE PASSAGE IN TISSUE CULTURE

Subject No	Days After Contact	$TCID_{50}$ Per 0 1 Ml	Ratio of Polio-Infected Monkeys: Intracerebral	Intraspinal	
			Undiluted	Undiluted	1·10
35	5	6 75	0/2	0/2	1/2
2	10	7 15	0/2	0/2	0/2
43	10	7 00	0/2	0/2	0/2
8	20	6 75	0/2	0/2	0/2
43	50	6 95	0/2	0/2	1/2

after 50 days induced no clinical symptoms when inoculated intracerebrally; when an inoculum diluted to 10^{-1} was injected intraspinally, 1 monkey out of 4 became paralyzed after 24 hours, but that the effect was due to poliomyelitis infection was questionable as judged by histologic examination Thus, of a total of 22 monkeys inoculated intracerebrally, none showed any clinical symptoms and only one showed histologic polio lesions.

Of the 44 monkeys inoculated intraspinally, 4 showed paralytic symptoms Histologic examination showed that in one monkey these symptoms were questionable and we therefore consider this paralysis as traumatic Histological examination indicated that 5 of those 44 monkeys showed changes typical for polio

Spread of Type 1 virus in non-vaccinated adult population of Wyszkow

For several weeks before vaccination of children in Wyszkow, stool specimens were collected from 398 individuals and tested for the presence of polio virus through inoculation of HeLa cell tissue cultures Two weeks after vaccination was completed, stools were again collected from the members of families that had been in direct contact with a vaccinated child, and from persons who had had no known contact with those receiving virus. Stools were again collected from these two groups 3 months later (Results of this study are shown in Table 13.) Type 1 polio virus was isolated from the stools of 5 subjects out of 398 specimens tested Three of the excretors were siblings The same number of subjects was found to excrete Type 2 virus and again three siblings were involved Seven subjects excreted cytopathogenic viruses not neutralized by any of the polio-immune sera. This low incidence of spread of polio Type 1 virus in the Wyszkow community was maintained throughout the 3 months observation period following vaccination with the attenuated strain. No differences were observed between the group of subjects in immediate contact with the immunized children and those who were supposed to have no contact at all.

Discussion

In planning these experiments with the CHAT strain, we tried to create conditions as similar as possible to those under which we would eventually have to carry out mass vaccinations

Plotkin, Koprowski, Richardson, and Stokes,[2] in their field trials with families in Moorestown, showed that the CHAT strain of attenuated polio virus was isolated in 100 per cent of vaccinated children (18/18). In unvaccinated children who were in contact with the vaccinated ones, virus was isolated in 37 4 per cent (13/35), and in parents living in contact with their vaccinated children, virus was isolated in 8 5 per cent (3/36).

Buser and Schar's studies with the same strain[3] showed that virus was isolated in 100 per cent of newborn babies and children who had no antibodies (1.5) before vaccination, in children with antibodies (titer 1·5 to 1:50), virus was isolated in 86 per cent, and in those with

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antibodies (titer 1:50 and over), in 64 per cent Among adults vaccinated with live vaccine and having antibodies (titer 1 50 and over), the percentage of those excreting virus was 23 per cent Gard[4] isolated CHAT virus in 100 per cent of vaccinated children in families The above results are seen to be almost identical with ours.

In our studies the percentage of children in whom virus was isolated ranged from 72 to 86 per cent, but we must take into consideration the fact that most of the vaccinees had pre-existing antibodies. The number of positive results in the fecal excretion of virus in our studies is similar to results obtained by Buser and Schar[5] in experiments carried out on children with pre-existing antibodies In the Wyszkow boys who shared living quarters with vaccinated school mates, virus was isolated in 16 6 per cent

In families of the children vaccinated in Warsaw, the virus was isolated from the feces of 29 per cent of the adults tested. This percentage was higher than that obtained by Koprowski *et al* (8 5 per cent) The difference may be accounted for by overcrowded houses and the resulting close and continuous contacts in the present study

If we take into consideration that the virus titer in feces ranges from 10^2 to 10^6 per gram, we may draw the conclusion that the CHAT strain has the ability to multiply in the intestines of vaccinated persons.

According to Koprowski[3] and Sabin,[6] attenuated virus multiplies well in subjects who have no pre-existing antibodies On the other hand, in people with pre-existing antibodies as a result of natural infection, the propagation and excretion of virus occurs in a low percentage. Verlinde[7] supposes that this difference is due not only to humoral resistance but also to the age of the individuals Adults having pre-existing polio antibodies excrete virus less readily than do children with similar antibodies

In this study, the CHAT strain administered orally did not induce clinical symptoms; but did multiply in the intestinal tract of people who had no pre-existing antibodies and also many of those with pre-existing antibodies. Ninety per cent of triple-negative individuals, and 90 per cent of those without Type 1 antibodies, developed Type 1 antibodies two months after administration of live vaccine. The titer of those antibodies was over 1·64 in more than 50 per cent of the individuals Vaccinated individuals with pre-existing antibodies showed a great increase of those antibodies· in 70 per cent of the vaccinees the increase was four-fold or greater. These results show that the vaccine has adequate immunizing properties.

The possibility that strains used for vaccination may eventually increase in virulence is now the subject of much discussion. The view has been advanced that strains may acquire virulence in passage through man's intestines and become the source of an epidemic in the community

Our studies showed that after two passages in man, the CHAT strain did not acquire enhanced monkey virulence Similar results were obtained by Koprowski[8] in 6 serial passages carried out in children Moreover, about 83 per cent of the so-called wild Type 1 strains circulating in nature, as was shown by Sabin's research, have high neuropathogenicity on intracerebral injection in the monkey Hence, during an interepidemic period, there are strains in the population with greater virulence than those used in the preparation of live vaccine

Therefore, in respect to an increased virulence of the virus, we think that the application of live vaccine should not present any danger

Conclusions

As a result of our studies on live poliomyelitis vaccine prepared from the CHAT strain by Dr. Koprowski, we draw the following conclusions

(1) CHAT strain does not induce any clinical symptoms when administered to man.

(2) It shows a capacity to multiply in the intestines

(3) It does not spread very easily to non vaccinated individuals.

(4) It has immunizing properties, causing the development of antibodies, or a considerable increase in the titer of antibodies.

(5) Poliovirus isolated from feces after vaccination with CHAT strain does not show any pronounced neuropathogenic properties as compared with the primary strain.

On the basis of the foregoing evidence, it appears that this strain can be used for mass vaccination Only further epidemiologic observations can enlighten us as to the efficacy of its immunizing properties

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DISCUSSION

Chairman Rhodes The paper presented by Dr. Przesmycki is open for discussion.

Dr. Koprowski. I would like to call your attention to the fact that the same pool of Type 1 virus which in the Leopoldville study has converted only 60 per cent of the children fed from sera negatives to sera positives, in the hands of Dr Przesmycki in Poland has induced formation of antibodies in 90 per cent of the subjects fed the virus Obviously more extensive studies have to be conducted in the Belgian Congo in order to evaluate the low sera-conversion ratio However, there is strong indirect indication that we are dealing here with interference by enteroviruses other than the one fed

Dr. Verlinde I would like to comment briefly on the results obtained by Dr. Przesmycki I was surprised by the absence of neurovirulence of the CHAT strain, which certainly is in contrast with findings by many others, and by myself, on the neurovirulence of attenuated strains

I recall the difference which we found between the paralytic attack rate of the Sabin strains of attenuated poliovirus when inoculated between the third or the fourth intervertebral space above the level of the iliac crests. Even when inoculated through the third space, which is certainly less favorable than through the fourth space, paralysis may occur In the absence of paralysis we found lesions regularly.

I wonder whether the discrepancy between the results of Dr Przesmycki and others can be explained by differences in the techniques of intraspinal inoculation

Dr. Sabin: May I address my question particularly to Dr. Koprowski and Dr Przesmycki One of the things I was intrigued by was the high incidence of virus isolation from the children who were naturally immune, who had spontaneous Type 1 antibody

As I recall, Dr. Koprowski's previous reports were quite in line with those of others in which the incidence of isolation from such individuals was very much lower than from those without any demonstrable antibody Now here they were very high.

I assume that Dr. Przesmycki had time to type all the isolates, and that they were all actually Type 1; and if they were Type 1, I wonder if we could have some comment on this rather unusual manifestation.

Was it merely a small amount of virus on an occasional isolation, or what is the picture, really?

Dr Koprowski: In the study reported by Dr Przesmycki, all fecal virus samples were identified in a neutralization test There is perhaps a higher than usual incidence of isolation of the fecal virus from children who were naturally immune before being fed the virus In most cases more than one stool sample showed the presence of the virus However, I do not recall exactly what was the concentration of the virus in the feces of those who had antibodies prior to feeding, as compared to those who did not

Dr Verlinde said that he was surprised "by the absence of neurovirulence of the CHAT strain, which certainly is in contrast with findings by many others, and by myself, on the neurovirulence of attenuated strains" I am quite sure that Dr Verlinde did not have the CHAT strain in his laboratory and that he can only make reference to the strains that were available to him for his own observations Dr Przesmycki is responsible for the data he obtained, and Dr Verlinde for his results If the difference in techniques in the intraspinal inoculation in monkeys yields such greatly different results, then it is my opinion that we should not pay great attention to the intraspinal inoculation unless and until we find a strain which will give no pathogenic lesions after inoculation through this route, regardless of the "delicacy" of the technique employed We should examine rather carefully the results obtained by Dr Przesmycki and others with intracerebral pathogenicity of the CHAT strain after one or more passages through man. Here the differences in technique cannot account for the striking fact that in the hands of Dr Przesmycki, Gard, and ourselves

the first human passage material does not indicate any increase in the pathogenicity for monkeys injected intracerebrally This may be a characteristic of the strain and we are investigating this further.

DR GARD: I would like to make a remark in relation to Dr. Sabin's question.

I hope that I made it clear yesterday that the results of the feeding of virus to natural immunes seemed to depend upon the previous history of those immunes.

Whereas 30 adults with natural immunity at the start of our trial proved highly resistant—only three of those became infected when given virus orally—there were 26 children who had excreted virus on first feeding and, as far as we could judge, had been exposed to live virus just this once Among those 26 children, 21 became excreters on re-feeding less than ten months after the first infection.

Therefore, depending upon the previous experience of the population of "naturally immunes" you are dealing with, you might expect differences in results

12. VACCINATION WITH ATTENUATED POLIOVIRUSES IN COSTA RICA

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DR QUIRCE *(presenting the paper)* On 16 March 1959 Costa Rica embarked upon a nation-wide program of vaccination with live attenuated poliovirus vaccine

A cursory glance at the history of poliomyelitis in Costa Rica in recent years will quickly reveal the reasons for the interest in controlling this disease (Table 1) In 1954 the country suffered one of the most severe epidemics of Type 1 poliomyelitis in medical history In a population of approximately one million inhabitants there were 1,081 cases of paralytic poliomyelitis Since that time, the endemic level of the disease has risen steadily with a smaller epidemic occurring again in 1956 A vaccination program with Salk vaccine was carried out in 1956-58, but was gradually diminished when it became clear that the economic resources of the country could not support an adequate campaign with this vaccine

Toward the end of 1958, therefore, the Ministry of Health requested the advice and support of the Pan American Health Organization in conducting a live poliovirus vaccination program A plan was adopted to vaccinate all children under 11 years of age in the country, beginning with the metropolitan area of San José, the capital of Costa Rica.

The program is being carried out by giving first the Type 2 virus, followed by Type 1 and Type 3 at approximately one month intervals Since 10 May, in addition to the Ministry of Health's personnel, the full-time assistance of three epidemiologists has been provided from the PASB, as well as laboratory support from the Middle America Research Unit in Panama, and the PASB Tissue Culture Laboratory at Cali, Colombia Surveillance of illness possibly related to vaccination is receiving primary attention. In addition, serological surveys are being conducted before and after vaccination, and the antibody responses of newborn infants are being studied.

To 12 June 1959, 80,912 doses of Type 2 and 67,594 doses of Type 1 vaccine have been admin

TABLE 1 AGE DISTRIBUTION OF REPORTED CASES OF POLIOMYELITIS FOR SELECTED YEARS, COSTA RICA, CENTRAL AMERICA

YEAR	AGE (NUMBER)							
	TOTAL	−1	1–4	5–14	15–24	25–44	45+	UNK
1941	24	4	18	2	—	—	—	—
1944	84	5	56	23	—	—	—	—
1950–51	37	11	20	6	—	—	—	—
1954	*1,081*	*215*	*710*	*106*	*36*	*8*	*1*	*5*
1955	45	4	28	7	5	—	1	—
1956	170	31	112	16	5	4	1	1
1957	51	8	29	8	3	—	1	2
1958	62	14	34	7	1	1	2	3

* Dr Quirce (Minister of Public Health, Costa Rica); Dr Vargas Méndez (Director General of [illegible]); Dr Núñez (Ministry of [illegible] fender- [illegible] anitary [illegible] erican

istered Vaccination with Type 3 virus is beginning Forty vaccinators are employed in a house-to-house program The vaccine is dispensed in a sweetened cherry-flavored solution by medicine droppers into plastic spoons and fed to the children. The vaccine is accepted readily by the children The dose is approximately 0 7 cc. of vaccine equivalent to $10^{5.8}$ and $10^{6.7}$ TCD_{50} of Type 2 virus (depending on the lot used) and $10^{6.3}$ TCD_{50} of Type 1 virus

Vaccination in the metropolitan area of San José will be completed within six weeks, at which time the program will be extended to the rest of the country

With the poliomyelitis consciousness developed as a result of the 1954 epidemic, the reporting of poliomyelitis and related diseases has improved considerably in Costa Rica Suspect cases of poliomyelitis are referred to the San Juan de Dios Hospital, which has a large infectious disease section and a modern rehabilitation unit

Since the onset of the program on 16 March, all cases of suspect poliomyelitis regardless of vaccination status have been investigated, along with all reported reactions to the vaccine In addition, 202 families in the metropolitan area of San José were surveyed in order to determine unreported polio-like illness

To date, no definite clinical cases of paralytic poliomyelitis have been detected among vaccinated children There were two instances of central nervous system illness among vaccinated children, which are under study One case of transient leg weakness with no other symptoms 30 days following ingestion of Type 2 vaccine has yielded Type 1 poliovirus and possibly another viral agent The second case had fever and meningismus of 36 hours duration, 5 days after Type 1 vaccination No viral agents were isolated from throat and rectal swabs inoculated into monkey kidney tissue culture and suckling mice

Another group of 15 vaccinated children with central nervous system illness has been studied and the diagnosis of paralytic poliomyelitis was ruled out These include one suspect case of tuberculous meningitis, a cerebellar lesion, an anesthesia complication, isolated weakness of the buccinator muscle, two cases of status epilepticus, two febrile illnesses with convulsions, three bacterial meningitides, and a psoas abscess

A large number of cases of minor illnesses has been reported by families as possible reactions to the vaccine These have included febrile respiratory and gastrointestinal complaints which fall into no particular pattern and in the opinion of the investigating team do not differ from illnesses seen among non-vaccinated children A number of allergic skin reactions has been reported, four of which could be related to vaccination. These are currently being investigated.

In the survey to determine unreported illness 1,101 individuals in 202 families were interviewed Illness requiring bed rest for longer than 24 hours during the previous month was reported in 62 instances A physician saw 54 of these cases. There was no instance of paralytic disease There were two cases of fever and neck pain in one family These children (both of whom had received 3 doses of Salk vaccine) were not vaccinated with the oral vaccine, but vaccination was carried out in the vicinity of their home

To date, in the metropolitan area of San Jose, no cases of paralytic poliomyelitis have been reported in the families of vaccinated children However, eight cases of suspect poliomyelitis developed among unvaccinated individuals during the vaccination program These included one case of flaccid paralysis occurring during an attack of mumps, a case of sudden weakness of all extremities with no other symptoms, two cases with fever and neck pain, referred to previously, and four cases in which the prominent symptoms were those of meningitis and spasticity No viral agents have yet been isolated from available specimens inoculated into monkey kidney and HeLa cell cultures as well as suckling mice

Among 24 reported suspect poliomyelitis cases in individuals residing outside of the zone of vaccination, four were clinical poliomyelitis, six were possible poliomyelitis, and 14 were cases in which a diagnosis of poliomyelitis was excluded

Serological Survey of Poliovirus Antibody

In the original program it was planned to collect blood samples from one per cent of the vaccinated population before vaccination and four to six weeks after the last dose Because of administrative difficulties, only 450 blood specimens were collected from the metropolitan area

TABLE 2. DISTRIBUTION OF POLIOVIRUS ANTIBODY IN SINGLE SPFCIMENS AND ALL SPECIMENS BY SEROTYPE AND AGE BEFORE LIVE POLIOVIRUS VACCINATION—SAN JOSF, COSTA RICA, 1959

Classification of Single Specimens by Serotype	6-11 Mo		1		2		3		4		5-9		10-15		Adult*		Total
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
In Single Specimens																	
None	7	78	12	38	7	17	0	0	2	3	1	1	0	0	1	1	30
1, 2, 3	0	0	1	3	6	15	22	43	43	74	156	86	24	89	123	80	375
1, 2	0	0	0	0	4	10	7	14	2	4	7	4	2	7	14	9	36
1, 3	0	0	0	0	0	0	1	2	0	0	6	3	0	0	3	2	10
2, 3	0	0	3	9	12	30	14	27	6	10	8	4	1	4	6	4	50
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
2	0	0	7	22	5	13	4	8	1	2	2	1	0	0	6	4	25
3	2	22	9	28	6	15	3	6	4	7	2	1	0	0	0	0	26
Total	9	100	32	100	40	100	51	100	58	100	182	100	27	100	154	100	553
Classification of All Specimens by Serotype — In All Specimens																	
None	7	78	12	38	7	17	0	0	2	3	1	1	0	0	1	1	30
1, 2, 3	0	0	1	3	6	15	22	43	43	74	156	86	24	89	123	80	375
1	0	0	1	3	10	25	30	49	45	78	169	93	26	96	141	92	422
2	0	0	11	34	27	68	47	92	52	90	173	95	27	100	149	97	486
3	2	22	13	41	24	60	40	78	53	91	172	94	25	93	132	86	461
Number of specimen	9		32		40		51		58		182		27		154		

* Cord blood.

of San José. Of these, approximately one third had received Salk vaccine In order to determine whether these specimens were truly representative of the population, a graded random survey was conducted by region. Several readily determined factors relating to the socio-economic status of the 131 families bled and 202 control families were compared From the preliminary results of this study it became obvious that the test group does not represent a true random sample of the population of San José, because of a predominance of families in the lower socio-economic condition. The data summarizing the distribution of antibodies in the population before vaccination are presented in Table 2.

An additional phase of the program includes the collection of cord blood samples from babies born in hospitals, and the feeding of a combination of Types 2 and 3 vaccine 48 hours after birth. Type 1 virus is being fed one month later At six months of age the infants will be bled again

In summary Costa Rica is engaged in the first nation-wide program in the Americas with the attenuated poliovirus vaccine, aimed at immunizing the population under 11 years of age. The program is approximately two-thirds completed in the metropolitan area of San José The acceptance of the vaccine on the part of the population has been almost universal. No untoward incident implicating the safety of the vaccine has been encountered The evaluation of the effectiveness of this program in reducing or eliminating paralytic poliomyelitis will be a problem for the future.

The vaccine strains being used in the program —SM, Type 1, MEF₁, Type 2 and Fox, Type 3— were provided by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., through an agreement with PASB

The serum antibody determinations were carried out by Microbiological Associates, Inc., in Bethesda, Maryland, U.S.A.

DISCUSSION

CHAIRMAN RHODES: The paper presented by Dr Quirce is open for discussion

DR LANGMUIR I did have the unusual opportunity of being invited by the Pan American Sanitary Bureau as a temporary adviser to accompany Dr Henderson and Dr Brody, along with Dr Shelokov from Panama, on a visit to Costa Rica to discuss the whole problem of the surveillance of this program

I think everyone is aware that I am deeply concerned about the safety problem

I suspect that those of us who lived through the fires of the Cutter incident are more sensitive, just as those of us who lived through the depression years, who were hungry, have a little more respect for what can happen in the economic cycle

I believe deeply that one must have a very fine screen of what goes on, and just the number of reported cases, or even the number of hospital admissions, of paralysis is totally insufficient as an adequate surveillance of one of these problems. We have attenuated strains being fed, and it is quite possible, it seems to me, that patients with relatively minor illnesses, patients with relatively minor degrees of paralysis may follow, and unless there is a thorough and constant search for such cases, they could well be missed

Such an eventuality might be faced when live virus vaccines are given in other parts of the world, such as in northern Europe, with a largely triple negative population A series of cases might very well produce another type of Cutter incident.

I was in Costa Rica only four days There are several others in the room who have been much more active in the continuity of this surveillance program. It is not an easy thing to set up, and I do believe this deserves careful discussion, because a thorough search for cases is essential in any adequate epidemiological evaluation of safety

DR. DICK: I would like to emphasize what Dr Langmuir has said, that we are still trying to establish safety and efficacy.

While in many countries a calculated risk may be taken under certain conditions, at the same time I think we must remember that for other countries we have to have evidence, and I appreciate that this is a difficult thing to do. I am saying this now only to indicate that we are still measuring safety and efficacy in other countries

We have to have the assurance that what is being used will be as safe and as efficient as the Salk vaccination.

One other point that I find difficult to understand is the comparison in the interference which occurs with other enteroviruses, in Dr Sabin's vaccine, and other enteroviruses, and Dr. Cox' vaccine I think that is worthy of a certain amount of discussion at this stage

Finally, I would just like to say that for many years, up to now, cholera vaccination has been used in many parts of the world, used by people who believe that it is effective, and we do not yet know, really, whether it is an effective vaccine The implications of that are very clear

DR COX These problems of interference which Dr Dick has raised are quite important I can only repeat what I said this morning that when we analyze the data from Andes, Colombia, and that obtained in Minnesota, which perhaps has a population relatively clean of enteroviruses in comparison to Colombia, we have found the immune responses to Types 1 and 3 vaccines to be almost identical Responses to Type 2 were somewhat lower in Colombia than in Minnesota, but that may have been because in Andes, but not in Minnesota, Type 1 was fed first and it may have interfered with the Type 2 vaccine, since the Type 2 appears to be the least invasive of the three strains with which we are working

Naturally, we hope to get the answer regarding interference to our strains by other enteroviruses in the Costa Rica trial, and I believe we will

DR HENDERSON Having been involved in the surveillance aspect of the program in Costa Rica, I would like to add that we found it exceptionally difficult to carry out this phase of the

program; that the more active the search for possible cases became, the more difficult it was to know what we were dealing with Among others, we uncovered a number of limping children and children with stiffness of the neck. We found it a problem to work up each of these cases in complete detail, and yet where should we draw the line?

Even when we had the laboratory data, it was not easy. Dr. Shelokov and I spent considerable time trying to interpret particular isolates and deciding what the interpretation would be if we did or did not get particular antibody responses

For example, there was an unvaccinated two-year old near San José who developed paralytic poliomyelitis, and from whom we isolated a Type 2 virus from the stool. We do not know whether this was a wild strain or a vaccine strain. Serology will not help Since, as Dr Dick says, "it is not what goes in that is important, it is what comes out," we can only consider this to be a possible vaccine-related case.

A second case was a seven year-old in San José who was not vaccinated He developed mumps, followed a week later by flaccid paralysis At this moment the stool specimens are negative If we get an antibody response for polio, does this mean he had mumps and polio, or does this indicate incidental intestinal polio infection in an area where the virus has been heavily seeded? I do not know how we can interpret it.

These are but two of the cases we found There are many more

In addition to what is listed here in terms of number of cases, I think we saw on the order of 80 to 100 additional individuals, and for us, this relatively small-scale project was a full time job for one physician and a part-time job for another

Dr Paul An interesting point about this report, already commented upon by two discussants, concerns methods of surveillance Yesterday, in a brief comment, I expressed the hope that, as we viewed these various field trials and the manner in which they were surveyed, some *approved standards* should be recognized for the assessment of the safety and effectiveness of these vaccines.

There is a point about the enteroviruses which we ought to remember, namely, that some enteroviruses are interferers, as we heard in the paper from Poland this morning; others may not be. We have heard, for instance, from Dr. Fox and his group, as to how ECHO 1, ECHO 9, and, I think, Coxsackie B5 may be incriminated

We must remember that these interfering infections are not always there. They come in waves, and what is true in 1959 may not be true in 1960

Dr Montoya. I would like to emphasize the difficulties of surveillance, but also to point out that in Costa Rica, fortunately, there are conditions indicating that such a study could be done well. The country is small and has in the capital one large central hospital which receives more patients by plane, from the other states, than any other place in the world. Also, it has a fairly adequate number of physicians giving a rather complete medical coverage to the population For those reasons I believe that although it is a full time job, and quite a difficult one, Costa Rica offers good opportunities for successful work

Dr Payne: In considering the question of safety, there is one factor which has not, I believe, been mentioned at all, and that is the problem of provocation poliomyelitis One of WHO's field teams had an unfortunate experience in Western Samoa, in which repository penicillin was being used in a yaws control program

There was an epidemic of poliomyelitis comprising 25 cases, of which 24 cases were pure provocation poliomyelitis In each case the paralysis was either confined to or began in the injected limb

If this campaign had not been going on, there would have been presumably just one case of poliomyelitis due to the prevalent strain, which was apparently not highly paralytogenic

I believe that if we are feeding live virus, we have to bear the possibility in mind that some form of provocation might make these viruses more likely to cause symptoms

Dr Montoya: I would like to clarify the remarks made by Dr Henderson regarding the difficulties of surveillance encountered in San José. It is important to become acquainted with the morbidity picture in the country at this time

of the year—with epidemics of measles and mumps going on—in order to be able to exclude many apparent associations with the vaccine I am sure that if one were to carry out a surveillance program for some other problem in other parts of the country at this time, or even in San José after the vaccination program has been completed, one would encounter the same findings, if not worse. So, we must take into account what is going on currently in the country, and not necessarily relate the findings from a single sampling to the vaccine.

13. PRELIMINARY REPORT ON MASS ORAL IMMUNIZATION OF POPULATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINE FROM A.B. SABIN'S ATTENUATED STRAINS

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DR VOROSHILOVA *(presenting the paper)* Prophylactic Salk vaccine against poliomyelitis prepared from formalin-killed virus was the first great achievement in the field of broad specific poliomyelitis prophylaxis The significance of this achievement can hardly be overestimated and we are grateful to Dr Jonas Salk for his great contribution to modern medicine

At present, however, with all its well known merits, Salk vaccine cannot be considered quite satisfactory for certain countries for the eradication of epidemic poliomyelitis It does not give protection from paralysis to a relatively large proportion of vaccinees—up to 20%-30% Poliomyelitis virus can multiply in those vaccinated with killed virus vaccine Postvaccinal immunity is not complete and does not produce enough resistance in the portals of entry of infection. Epidemic poliovirus continues to circulate among the population and gives menace of new poliomyelitis outbreaks Production of killed-virus vaccine of high potency and effective control of its safety demand very great efforts and considerable expenditure, which restrains the rate of development of production of this preparation and does not permit giving protection to all who need it in proper time

The method of intramuscular or subcutaneous injection of the killed virus vaccine is not very convenient for the repeated mass inoculations to many dozens of millions of people that are required for the creation of immunity to poliomyelitis in entire populations

All this stimulates the search for new ways of perfecting specific poliomyelitis prophylaxis

The application of live virus vaccines seems to us most promising

Experimental investigations and epidemiological and clinical observations by A B Sabin, H Koprowski and co-workers, Cox and co-workers, A A. Smorodintsev and co-workers, and others, have given solid theoretical foundation for the development of suitable methods and for large-scale tests of live virus vaccine from attenuated strains of poliovirus

Doctor A B Sabin has successfully selected plaque-purified attenuated vaccine strains of three types of poliovirus which meet the special requirements of the Expert Committee on Poliomyelitis, World Health Organization (1957)

At our request, Dr Sabin in September 1958 gave us about 110 thousand doses of each of these strains (from a large lot of his vaccine prepared in the U S A in December 1956).

We take this opportunity to express our deep gratitude to Dr Sabin for giving us vaccination material and for the very valuable, helpful advice and the attention to our work

The material obtained from Dr Sabin was used for immunization of a little more than 27 thousand people in the Esthonian and Lithuanian Republics Besides that, using Sabin's vaccine as seed virus, we prepared a large lot of live virus vaccine from Sabin's three strains, enough for the immunization of 10 million people (more than 100 liters of each of the 3 types) The vaccine from Sabin's strains was prepared in our Institute by growing the vaccine viruses taken directly from the author's vials in monolayer monkey kidney cell cultures, or from the first passage of these strains The characteristics of a large lot of live virus vaccine prepared in our Institute and its comparison with Sabin's original vaccine will be presented in a separate report

Starting the investigation of immunologic and epidemiologic effectiveness of oral immunization with live virus vaccine from Sabin's strains, we considered the problem of safety of Sabin's strains settled, because in 1957-1958 there were some highly convincing observations carried out

in a number of countries, first of all observations by Dr Sabin himself

Indeed, observations by Professor A. A Smorodintsev and co-workers in Leningrad, established complete innocuity of oral administration of Sabin's strains to about 1,200 children under 3 years Czechoslovakian experts, V. Skovránek, K. Zácek, and others, in December 1958 carried out successfully oral immunization of over 143 thousand children with Sabin's vaccine Observations by Prof Verlinde in Leiden (1957), Dr Ramos-Alvarez and Prof F. Gómez in Mexico (1957, 1958), Prof James Hale of Malaya University in Singapore—in about 200,000 children—left no doubts in the safety of orally administered Sabin's attenuated poliovirus strains Accordingly, we had nothing to risk when we started our investigations on the effectiveness of mass oral immunization with the vaccine from Sabin's strains

However, upon decision of the Presidium of the Academy of Medical Sciences, USSR, we were to broaden contingents of observation gradually, starting immunization in Esthonia for about 26,000 people and then broadened the scale of immunization because we were able again to confirm the safety and absence of any reactions to oral immunization

The first cycle of immunization with Sabin's original vaccine in Esthonia was carried out in January-March 1959 Table 1 shows data on age distribution of the vaccinated with Sabin's original vaccine in Esthonia

TABLE 1. AGE DISTRIBUTION OF VACCINATED WITH SABIN'S VACCINE IN THE ESTHONIAN SSR

AGE-GROUP	NUMBER VACCINATED	%
0 — 6 mo	299	1 2
6 m — 1 y	817	3 2
1 — 2 y	1,009	4 0
2 — 3 y	1,019	4 0
4 5 y	1,778	7 1
6 — 7 y.	2,335	9 3
8 —10 y	4,550	18 2
11 —15 y	6,162	24 4
16 —18 y	2,034	8 3
18 +—	5,107	20 3
Calculated by ages	25,160	100 0
Not calculated	1,174	
Total vaccinated	26,334	

Close clinical observation of the vaccinees showed no cases of central nervous system disease which could be associated with the vaccination

There were no cases of fever, or local or general reactions The vaccine proved to be completely areactive, harmless, and immunologically effective by laboratory data

Favorable results of the first vaccination cycle led local health offices to the decision to increase the scale of live virus vaccination. This was helped by conviction in unfavorable prognosis for 1959 because of rather high poliomyelitis incidence in 1958 in some Baltic areas

Upon request from local health offices our Institute supplied them with necessary amounts of vaccine prepared in Moscow from Sabin's attenuated strains and helped to organize mass vaccinations in the Esthonian, Lithuanian, and Kazakh Republics.

In the spring of 1959 in Esthonia most broad oral immunization has been carried out more than 694,000 persons aged from 2 months to 50 years received orally three types of poliovirus vaccine Table 2 presents data on age distribution of 639,444 persons vaccinated in the Esthonian SSR The table also shows per cent of vaccinated in given age groups for the whole republic and for the towns of Tallinn and Tartoo

The data presented in Table 2 show that considerable proportions of population in the most susceptible age groups were vaccinated Because of the peculiar age incidence of poliomyelitis in Esthonia in 1958, when peak incidence was in the age group 20-30 years, it was decided to vaccinate in 1959 older age groups, this caused a considerable increase in numbers of vaccinated in the age group 20 50 years

Up to 10 June 1959, a total of 694,444 persons were vaccinated with live virus vaccine in Esthonia, including 26,334 vaccinated with Sabin's original vaccine and 666,110 vaccinated with the vaccine prepared from Sabin's strains in Moscow

In the Lithuanian SSR, vaccinations were given mainly to people in age groups from 2 months to 15-20 years Up to 27 May 1959, 546,952 persons received the vaccine.

In the Kazakh SSR the number of vaccinated up to 10 June 1959, was 1,050,450 persons The age of the vaccinated was from 2 months to 15 years. These data are presented in Table 3

TABLE 2 SCOPE OF VACCINATION WITH LIVE VIRUS VACCINE OF THE POPULATION IN ESTHONIAN SS[illegible] BY AGE GROUPS (UP TO MAY 10, 1959)

AGE GROUP.	IN ESTHONIAN SSR		IN TALLINN		IN TARTOO	
0 — 6 m	3,829		1,020		259	
6 m — 1 y	9,167	57 1%	2,102	57 0%	644	51 0%
1 — 3 y	31,751		8,378		2,191	
4 — 6 y.	44,597		12,019		2,521	
7 — 9 y.	44,839		11,321		1,870	
10 —12 y	44,553	68 0%	11,529	64 0%	1,927	43 0%
13 —15 y.	35,552		7,110		1,632	
16 —20 y	58,832	54 0%	15,215	60 9%	4,692	71 0%
21 —30 y	138,400		37,981		11,810	
31 —40 y	122,611	50 4%	35,340	61 0%	9,582	59 0%
41 —50 y	102,314		29,092		6,743	
TOTAL	639,444		171,106		43,871	

% calculated for vaccinated in a given age group Only results of mass vaccination with the vaccine pr[illegible] pared at the Institute for poliomyelitis research are presented (vaccinations with Sabin's vaccine [illegible] included)

TABLE 3. NUMBER OF VACCINATED WITH LIVE POLIOVIRUS VACCINE (UP TO JUNE 10, 1959)

REPUBLICS	VACCINE	1ST VACCINATION	2ND VACCINATION	3-RD VACCINATIO[illegible]
Esthonia	Original	26,334	25,750	23,909
	Secondary	666,110	633,022*	603,260*
Lithuania	Original	1,547	1,541	1,350
	Secondary	546,952	455,484*	346,500*
Kazakhstan	Secondary	1,050,450*	350,000*	
TOTAL	Original	27,881	27,291	25,359
	Secondary	2,263,512	1,438,512	949,760

* Data incomplete

TABLE 4 SCOPE OF VACCINATION WITH SABIN'S VACCINE OF THE POPULATION IN LITHUANIAN SS[illegible] BY AGE GROUPS (UP TO MAY 10, 1959)

AGE GROUP	POPULATION (IN THOUSANDS)	NO VACCINATED (IN THOUSANDS)	% VACCINATED
0— 7	390	164	42
7—15	450	256	57
15—19	270	147	54
TOTAL	1,110	547	49 7

The data in Table 4 show that almost half of the susceptible population in the Lithuanian Republic received live virus vaccine

In the Esthonian SSR and the Lithuanian SSR, serologic surveys for poliomyelitis virus antibody were carried out [illegible]

and Table 6.

TABLE 5 ABSENCE OF POLIOMYELITIS VIRUS ANTIBODY IN SERA FROM POPULATION IN ESTHONIAN SSR (IN SERUM DILUTION 1,4, BY pH TEST. DOBROVA, I. N.)

Age (Years)	Total Number of Sera Tested	Number of Sera Without Antibody to						Including Sera Without Antibody to All Three Types	
		Type 1		Type 2		Type 3			
		No	%	No	%	No	%	No	%
0— 1	53	46	87	35*	67	46	87	—	—
1— 4	221	115	52	53	24	125†	56	73	26 6
4— 7	67	31	46	16	24	27	40	8	11 9
7—10	38	9	24	5	13	6	16	2	5 2
10—16	83	18	22	8	10	22	27	2	2 4
16—21	19	3	18	3	16	10	53	—	—
21—30	38	7	18	7	18	18	47	3	8 0
	519	229	44	127‡	25	264**	51	88	17 0

* Calculated for 52 sera (=35/52)
† Calculated for 222 sera (=125/222)
‡ Calculated for 518 sera (=127/518)
** Calculated for 520 sera (=264/520).

On the basis of data on per cent distribution of sera without antibody to Types 1, 2, and 3 poliovirus in age groups, there was calculated the number of orally vaccinated with live virus vaccine who had no detectable pre-vaccination antibody to one or another poliovirus type These data may be of interest as an indication of the safety of the live virus vaccine used, because one can see the number of persons completely susceptible to poliovirus who received oral immunization

TABLE 6 ABSENCE OF POLIOMYELITIS VIRUS ANTIBODY IN SERA FROM POPULATION IN LITHUANIAN SSR (BY pH TEST YANKEVICH, O D.)

Age (Years)	Total Number of Sera Tested	Number of Sera Without Antibody to						Including Sera Without Antibody to All Three Types	
		Type 1		Type 2		Type 3			
		No	%	No	%	No	%	No	%
0— 1	28	19	68	16	57	21	75	12	42 6
1— 4	368	161	44	138	37	209	56	62	16 8
4— 7	42	10	24	9	21	10	24	1	2 4
7—15	347	107	31	158	45	119	34	32	9 2
15—19	75	27	36	43	57	35	47	15	20 0
	860	324	37 6	364	42 3	394	45 8	122	14 2

TABLE 7. AGE DISTRIBUTION OF VACCINATED WITH LIVE POLIOVIRUS VACCINE IN THE ESTHONIAN AND LITHUANIAN REPUBLICS, WITH SPECIAL REFERENCE TO NUMBER OF PERSONS WITHOUT PREVACCINATION ANTIBODY (UP TO MAY 27, 1959)

AGE GROUP	TYPE 1		TYPE 2		TYPE 3	
	TOTAL NUMBER VACCINATED	WITHOUT ANTIBODY	TOTAL NUMBER VACCINATED	WITHOUT ANTIBODY	TOTAL NUMBER VACCINATED	WITHOUT ANTIBODY
2/12 mo — 1 y	41,329	30,471	16,511	9,706	34,219	27,122
1 — 4 y	94,631	44,479	36,989	12,756	82,946	46,450
4 — 7 y	123,939	40,461	48,797	10,572	108,977	33,725
7 —15 y	386,189	108,683	156,736	60,639	339,904	101,193
15 —19 y	189,165	54,984	78,177	38,086	165,259	81,510
21 —30 y	141,349	25,443	23,702	4,266	134,897	63,402
TOTAL	976,602	304,521	360,912	136,025	866,202	353,402

In the course of the mass vaccination campaign there was not a single case of central nervous system disease which could be associated with the vaccination. In connection with this, the data in Table 7 on the number of persons without detectable antibody before vaccination are convincing evidence in favor of the safety of the live virus vaccine from Sabin's strains.

According to incomplete data, the total number of persons susceptible to Type 1 poliovirus among the vaccinated was more than 304,000, susceptible to Type 2—136,000, and to Type 3—more than 353,000 persons. This table does not include data on vaccinated over 30 years of age because of the lack of complete data on per cent distribution of negative sera in this age group.

We hope that the data presented allow us to make final conclusion of the clinical and epidemiological safety of oral immunization with live poliovirus vaccine from Sabin's attenuated strains.

Serological and virological investigations

Epidemiologic effectiveness of oral immunization with live attenuated virus vaccine is at present the most urgent and least studied aspect of the problem of specific prophylaxis of poliomyelitis.

Information on epidemiologic effectiveness of the mass vaccination we carried out may be obtained only at the end of 1959, that is after the analysis of seasonal incidence of poliomyelitis in the vaccination areas.

The question of the epidemiologic effectiveness of this vaccine is to a great extent connected with the immunologic evidence of the rise of antibody after immunization with live attenuated virus. On the basis of serological data, a prognosis of the possible epidemiological effectiveness of vaccination with Sabin's vaccine may be made.

During large-scale trials of live virus vaccine from Sabin's strains in the Lithuanian, Esthonian and Kazakh SSR, blood samples (venous) were taken from persons of all ages, mainly from children under 7-8 years, before vaccination and 1-3 months after oral vaccination. Collected blood samples were tested for antibodies to the three types of poliovirus beginning with undiluted serum or 1:2, 1:4 dilution against 100 TCD_{50} of each type of the virus by pH test. The technique of pH test was that in general use. Besides the vaccinated, their close contacts who did not receive the vaccine were tested also.

Up to the present we had time to check by pH test only a comparatively small proportion of the collected samples (246 paired sera and 37 triple samples).

Some tables are presented below which show antibody response one month after oral administration of Type 1 monovaccine (Tables 8, 9, and 10). Besides that, the completeness of

serologic response one month after the first feeding and one month after completion of three feedings is compared (Tables 11, 12, and 13). Figure 1 shows graphically the dynamics of antibody titers to Types 1, 2, and 3 in children without detectable prevaccination antibody to the corresponding types. These children received one feeding of a mixture of monovaccines of the three types and were tested one month after the beginning of vaccination.

TABLE 8 DEVELOPMENT OF TYPE 1 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 1 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 1 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (35 7% negative sera)	Negative	34	26	76 4
	Titer 4— 32	20	15	75 0
	Titer 64—512	36	9	25 0
	Titer 512	5	1	20 0
Total		95	51	53 7
Previously unvaccinated with Salk vaccine 115 children (72 1% negative sera)	Negative	83	51	61 4
	Titer 4— 32	22	8	36 3
	Titer 64—512	10	2	20 0
Total		115	61	53 4
Total 210 children (55 7%) negative)		210	112	53 3

TABLE 9 DEVELOPMENT OF TYPE 2 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 2 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 2 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (20% negative sera)	Negative	19	12	63 0
	Titer 4— 32	38	20	52 6
	Titer 64—512	33	13	48 5
	Titer 512	5	0	0
Total		95	45	47 3
Previously unvaccinated with Salk vaccine 115 children (57 4% negative sera)	Negative	66	37	56 0
	Titer 4— 32	27	16	59 2
	Titer 64—512	19	6	31 5
	Titer 512	3	0	0
Total		115	59	51.3
Total 210 children (40 5% negative)		210	104	49 5

TABLE 10. DEVELOPMENT OF TYPE 3 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 3 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 3 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (72 1% negative sera)	Negative	40	5	12 5
	Titer 4— 32	35	7	20 0
	Titer 64—512	15	2	13 3
	Titer 512	5	0	0
Total		95	14	14 7
Previously unvaccinated with Salk vaccine 115 children (72 1% negative sera)	Negative	83	6	7 2
	Titer 4— 32	21	7	33 3
	Titer 64—512	11	0	0
Total		115	13	11 3
Total 210 children (58 5% negative)		210	27	12 8

The data on serological survey one month after oral immunization with Type 1 live virus vaccine need some comment This survey was carried out mainly in one closed nursery where children of 6 months to 3 years lived Four days before vaccination with Type 1 live vaccine one case of poliomyelitis was reported in this nursery This was proved to be caused by Type 2 poliovirus Virological tests with stool samples collected before vaccination revealed in about 18% cytopathogenic agent carriage, and about half of these agents were Type 2 poliovirus Consequently, in this institution at the time of Type 1 virus vaccination there was latent circulation of Type 2 poliovirus and perhaps of some other enteroviruses as yet unidentified This probably explains the somewhat lowered number of positive Type 1 responses because of interference between Type 1 vaccine virus and spontaneous Type 2 poliovirus

Tables 9 and 12 shows data on the appearance of Type 2 antibody after vaccination with Type 1 monovaccine, which is perhaps explained by the spontaneous Type 2 poliovirus carriage in this children's institution.

Despite the probable presence of a certain

TABLE 11 DEVELOPMENT OF TYPE 1 ANTIBODY IN 37 CHILDREN (UNDER 3 YEARS) WHO HAD NO TYPE 1 ANTIBODY BEFORE LIVE POLIOVIRUS VACCINATION (pH TEST)

APPEARANCE OF TYPE 1 ANTIBODY			
ONE MONTH AFTER TYPE 1 LIVE VIRUS VACCINE		AFTER COMPLETION OF 3 IMMUNIZATIONS OR 3 MONTHS AFTER TYPE 1 LIVE VIRUS VACCINE	
NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
34	25* 73 5%	37	37 100%

* In this children's institution before vaccination with Type 1 vaccine there was 1 case of poliomyelitis caused by Type 2 virus, and also 8 strains of this type of virus were isolated from normal carriers Therefore interference of these two viruses is possible

serologic response one month after the first feeding and one month after completion of three feedings is compared (Tables 11, 12, and 13) Figure 1 shows graphically the dynamics of antibody titers to Types 1, 2, and 3 in children with*out detectable prevaccination antibody to the* corresponding types. These children received one feeding of a mixture of monovaccines of the three types and were tested one month after the beginning of vaccination.

TABLE 8. DEVELOPMENT OF TYPE 1 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 1 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 1 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (35 7% negative sera)	Negative	34	26	76 4
	Titer 4— 32	20	15	75 0
	Titer 64—512	36	9	25 0
	Titer 512	5	1	20 0
Total		95	51	53 7
Previously unvaccinated with Salk vaccine 115 children (72 1% negative sera)	Negative	83	51	61 4
	Titer 4— 32	22	8	36 3
	Titer 64—512	10	2	20 0
Total		115	61	53 4
Total 210 children (55 7%) negative)		210	112	53 3

TABLE 9 DEVELOPMENT OF TYPE 2 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 2 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 2 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (20% negative sera)	Negative	19	12	63 0
	Titer 4— 32	38	20	52 6
	Titer 64—512	33	13	48 5
	Titer 512	5	0	0
Total		95	45	47 3
Previously unvaccinated with Salk vaccine 115 children (57 4% negative sera)	Negative	66	37	56 0
	Titer 4— 32	27	16	59 2
	Titer 64—512	19	6	31 5
	Titer 512	3	0	0
Total		115	59	51 3
Total 210 children (40 5% negative)		210	104	49 5

TABLE 10 DEVELOPMENT OF TYPE 3 ANTIBODY AFTER VACCINATION WITH TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) IN CHILDREN PREVIOUSLY VACCINATED WITH SALK VACCINE AND IN UNVACCINATED CHILDREN ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

GROUP	TYPE 3 ANTIBODY BEFORE LIVE VIRUS VACCINE	NO TESTED	APPEARANCE AND SIGNIFICANT RISE OF TYPE 3 ANTIBODY ONE MONTH AFTER FEEDING	
			NUMBER	%
Previously vaccinated with Salk vaccine 95 children (72.1% negative sera)	Negative	40	5	12.5
	Titer 4—32	35	7	20.0
	Titer 64—512	15	2	13.3
	Titer 512	5	0	0
Total		95	14	14.7
Previously unvaccinated with Salk vaccine 115 children (72.1% negative sera)	Negative	83	6	7.2
	Titer 4—32	21	7	33.3
	Titer 64—512	11	0	0
Total		115	13	11.3
Total 210 children (58.5% negative)		210	27	12.8

The data on serological survey one month after oral immunization with Type 1 live virus vaccine need some comment. This survey was carried out mainly in one closed nursery where children of 6 months to 3 years lived. Four days before vaccination with Type 1 live vaccine one case of poliomyelitis was reported in this nursery. This was proved to be caused by Type 2 poliovirus. Virological tests with stool samples collected before vaccination revealed in about 18% cytopathogenic agent carriage, and about half of these agents were Type 2 poliovirus. Consequently, in this institution at the time of Type 1 virus vaccination there was latent circulation of Type 2 poliovirus and perhaps of some other enteroviruses as yet unidentified. This probably explains the somewhat lowered number of positive Type 1 responses because of interference between Type 1 vaccine virus and spontaneous Type 2 poliovirus.

Tables 9 and 12 shows data on the appearance of Type 2 antibody after vaccination with Type 1 monovaccine which is perhaps explained by the spontaneous Type 2 poliovirus carriage in this children's institution.

Despite the probable presence of a certain

TABLE 11 DEVELOPMENT OF TYPE 1 ANTIBODY IN 37 CHILDREN (UNDER 3 YEARS) WHO HAD NO TYPE 1 ANTIBODY BEFORE LIVE POLIOVIRUS VACCINATION (pH TEST)

APPEARANCE OF TYPE 1 ANTIBODY			
ONE MONTH AFTER TYPE 1 LIVE VIRUS VACCINE		AFTER COMPLETION OF 3 IMMUNIZATIONS OR 3 MONTHS AFTER TYPE 1 LIVE VIRUS VACCINE	
NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
34	25* 73.5%	37	37 100%

* In this children's institution before vaccination with Type 1 vaccine there was 1 case of poliomyelitis caused by Type 2 virus, and also 8 strains of this type of virus were isolated from normal carriers. Therefore interference of these two viruses is possible.

TABLE 12 DEVELOPMENT OF TYPE 2 ANTIBODY IN 34 CHILDREN (UNDER 3 YEARS) WHO HAD NO TYPE 2 ANTIBODY BEFORE LIVE POLIOVIRUS VACCINATION (pH TEST)

APPEARANCE OF TYPE 2 ANTIBODY			
ONE MONTH AFTER TYPE 1 LIVE VIRUS VACCINE		AFTER COMPLETION OF 3 IMMUNIZATIONS OR ONE MONTH AFTER TYPE 2 LIVE VIRUS VACCINE	
NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
34	16* 47%	33	33 100%

* See footnote to Table 11

TABLE 13 DEVELOPMENT OF TYPE 3 ANTIBODY IN 34 CHILDREN (UNDER 3 YEARS) WHO HAD NO TYPE 3 ANTIBODY BEFORE LIVE POLIOVIRUS VACCINATION (pH TEST)

APPEARANCE OF TYPE 3 ANTIBODY			
ONE MONTH AFTER TYPE 1 LIVE VIRUS VACCINE		AFTER COMPLETION OF 3 IMMUNIZATIONS OR TWO MONTHS AFTER TYPE 3 LIVE VIRUS VACCINE	
NUMBER TESTED	NUMBER POSITIVE	NUMBER TESTED	NUMBER POSITIVE
32	1 3 1%	34	33 97 2%

extent of interference between Type 1 and Type 2 polioviruses, it may still be seen from Tables 11, 12, and 13 that one month after three feedings, some levelling of titers occurs and sufficiently frequent development of antibody to all three types takes place.

Consequently, the problem of interference of live vaccine with spontaneously occurring enteroviruses is of relatively little importance for the ultimate result of oral immunization Perhaps this may be explained by the massiveness of our oral immunization and by the influence of contagiousness of the vaccine virus, that is, by the additional effect of circulation of the vaccine virus in this population

We obtained very interesting results in trials with trivalent mixture of Types 1, 2, and 3 monovaccines (10^5 TCD of each type) It has been found that the great majority of children who before vaccination had no antibody to Types 1, 2, and 3 poliovirus develop antibody by the end of one month after oral immunization. Their antibody titers were quite comparable to the best results of separate immunization with monovaccines or with antibody titers after clinical infection This indicates the possibility of a certain

TABLE 14 COMPARATIVE DATA ON ANTIBODY TITERS ONE MONTH AFTER TYPE 1 VACCINE AND TRIVALENT VACCINE IN CHILDREN WITHOUT PREVACCINATION ANTIBODY TO CORRESPONDING TYPES OF POLIOVIRUSES

Serum Dilution After Immunization	Number of Sera With Indicated Antibody Titers					
	Type 1 Antibody		Type 2 Antibody		Type 3 Antibody	
	Children Without Prevaccination Type 1 Antibody		Children Without Prevaccination Type 2 Antibody		Children Without Prevaccination Type 3 Antibody	
	Type 1 Vaccine	Trivaccine	Type 1 Vaccine	Trivaccine	Type 1 Vaccine	Trivaccine
2048	1	1	1	1	—	2
1024	—	2	5	5	—	2
512	1	1	5	4	—	2
256	7	4	10	5	1	4
128	1	—	1	9	4	10
64	25	4	2	2	2	2
32	5	4	1	2	1	3
16	27	3	9	—	3	2
8	6	3	11	1	3	1
4	9	4	3	—	1	1
Total positive sera	82	26	48	29	11	29
Log_2geometric mean titer	4.9	5.5	5.9	7.7	4.4	6.6
Geometric mean titer	30	45	60	208	21	97

TABLE 15. DYNAMICS OF ANTIBODY TITERS IN 9 TRIPLE-NEGATIVE CHILDREN AFTER ORAL IMMUNIZATION WITH SABIN'S VACCINE (TYPES 1, 2, 3,)

	Before Immunization			After Type 1 Vaccine			After Three Feedings Types 1–3–2 Vac		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Ratio of positive sera	0/9	0/9	0/9	6/9	2/9	0/9	9/9	9/9	9/9
Log_2geometric mean titer	—	—	—	4.9	8.0	—	8.5	7.8	6.7
Geometric mean titer	—	—	—	23	256	—	362	223	104

simplification of the oral vaccination schedule and allows us in some cases to use single or double immunization with a mixture of the three vaccines under conditions when there is too little time left before the beginning of the usual poliomyelitis season

It is quite probable that after simultaneous oral administration of a sufficiently large dose of

Serum Dilution After Immunization	Type 1	Type 2	Type 3
2048+	o	o	oo
1024	oo	ooooo	oo
512	o	oooo	oo
256	oooo	ooooo	oooo
128	oooo	ooooooooo	oooooooooo
64	oooo	oo	oo
32	oooo	oo	ooo
16	ooo		oo
8	ooo	o	o
4	oooo		o
<4	ooo	o	
before immunization 4	*ooooooooo* oooooooooo oooooooooo 29	*oooooooooo* oooooooooo oooooooooo 30	*ooooooooo* oooooooooo oooooooooo 29
Geometric mean titer	45	208	97

Fig 1 Antibody response one month after oral administration of Sabin's trivalent poliovirus vaccine to children without prevaccination antibody (in serum dilution 1-4) to indicated types of poliovirus (by pH test)

vaccine viruses of all three types there arise favorable conditions for simultaneous multiplication in the intestinal wall of the three types of vaccine viruses without significant interference between them, which is frequently observed upon consecutive administration of vaccine viruses of different types. We may suppose that in the human alimentary tract there are enough susceptible cells suitable for vaccine virus multiplication. Another possible explanation of our positive results with trivalent vaccine may lie in the immunizing effect of the vaccine viruses circirculating after vaccination among susceptible children. That vaccine virus is contagious is a *proved fact*. Because of that, additional immunization occurs in those children who did not have enough antigenic stimulation in the first stage of vaccination

We paid great attention to the virological study of the dynamics of the vaccination process in persons orally immunized with live virus vaccine and in persons immunized by close contact with the vaccinated

Table 16 presents data on the comparative frequency of vaccine virus Type 1 isolation from feces of children under 3 years and of young adults 20-30 years As was expected, the incidence of virus excretion in children was very high—up to 97%, whereas among adults the virus could be isolated from stools in 19-21%, average 22%.

Duration of virus excretion in smaller children (see Table 17) was almost twice as long in persons aged 20-30 years

In those cases when before vaccination Type 2 poliovirus or untypable virus had been isolated from stools (Table 18), we could still observe the establishment of Type 1 virus, that is, it multiplied in the intestinal tract and then produced antibody to Type 1 Consequently, interference between Type 1 and Type 2 poliovirus is a temporary phenomenon having no significant influence on the ultimate result of vaccination

Preliminary data on the influence of oral immunization with live virus vaccine on poliomyelitis incidence †

According to records of poliomyelitis incidence in the Esthonian and Lithuanian SSR in June, July, and August 1959 there was no trend towards the usual seasonal rise in number of cases, on the contrary, there was some decrease in incidence These data may be considered as the first evidence of the epidemiologic effectiveness of live poliovirus vaccine.

† With addition of data as of 2 September 1959 on the incidence in June, July, and August.

TABLE 16 INCIDENCE OF ISOLATION OF VACCINE VIRUS FROM FECES

Previous Immunization With Salk Vaccine	Children 0—3 Years				Adults 20—30 Years			
	Vaccinated		Contacts		Vaccinated		Contacts	
	Number	%	Number	%	Number	%	Number	%
Yes	19/19	106	15	—	15/59	25	3/36	8
No	30/32	94	9/9	100	16/69	23	10/32	31
Total	49/51*	96	9/9	100	31/128	24	13/68	19
	58/60		97%		44/196		22%	

* 38 children among vaccinated (51 persons)—74%—had no Type 1 antibody before vaccination

Note This table does not include data on virus isolation from stools in those cases where virus was found in stools before vaccination.

TABLE 17 DURATION OF VACCINE VIRUS EXCRETION IN DIFFERENT GROUPS OF CHILDREN FROM 0 TO 3 YEARS

Groups by Presence of Antibody	Number of Persons Excreting Virus Up to Indicated Day						Average Duration of Excretion (in Days)	Intensity of Excretion*
	0	6	9	12	15	19		
1 − 2 − 3 − (20 pers)	—	2	—	1	6	11	16 1	4 5
1 + 2 + 3 + (8 pers)	1	2	1	4	—	—	8 6	1 5

* Average number of strains per 6 specimens.

TABLE 18 PRELIMINARY DATA ON ORAL IMMUNIZATION WITH TYPE 1 VACCINE OF CHILDREN—CARRIERS OF TYPE 2 POLIOVIRUS

AGE OF CHILDREN	ANTIBODY		VIROLOGIC INVESTIGATION ON DAYS							
			BEFORE		AFTER TYPE 1 VACCINATION					
	BEFORE	AFTER 1 MONTH	22	2	2	6	9	12	15	19
Yy 10 mo	ooo	64oo		Type 2	+	Type 1	+	+	+	−
Tr 17 mo	o64o	4, 1024	Type 2	Type 2	Type 2	−	+	+	−	−
Al 18 mo	+++		Type 2	Type 2	−	Type 1	+	−	+	−
Sh 22 mo	+++		Type 2	Type 2	Type 2	Type 1	+	+	+	−
Kr 21 mo (contact)	−++			Type 2				Type 1	−	−

TABLE 19 POLIOMYELITIS INCIDENCE IN ESTHONIA BY MONTHS

YEAR	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
1955	4	2	—	3	4	3	7	16	58	56	27	13	193
1956	12	5	1	3	8	10	15	39	45	65	27	12	236
1957	5	13	6	9	5	7	17	20	27	15	5	11	139
1958	8	2	6	8	4	5	20	151	407	266	90	24	991
1959	31	7	5	5	2	2	2	2	0				
Including vaccinated	—	—	—	1	1	1	2	2	—				

Ratio in J-J A S=88 6=14 6 (average for many years in J J A-S to the number of cases in the same months of 1959)

Declined by 93 2%

TABLE 20 POLIOMYELITIS INCIDENCE IN LITHUANIA BY MONTHS

YEAR	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
1955	1	1	6	3	5	20	46	116	106	58	31	31	427
1956	18	17	12	19	32	46	64	51	33	32	10	11	354
1957	12	9	12	9	6	19	37	23	21	8	4	12	172
1958	5	8	10	1	3	21	39	66	55	55	23	25	286
1959	10	10	8	4	3	3	8	2	2				
Including vaccinated (1959)	—	—	—	—	—	1	1	1	—				

Ratio in J-J-A-S=158 15=10 5.
Decline by 90 6%.

At present in the Lithuanian and Esthonian Republics, only sporadic cases of poliomyelitis were registered among those vaccinated orally with the vaccine from Sabin's strains There is every reason to consider these cases not to be associated with live virus feeding proper There was no accumulation of cases

Conclusions

On the basis of epidemiological, clinical and laboratory investigations carried out, a conclusion may be drawn of the safety of oral immunization with live vaccine from Dr. Sabin's attenuated strains of Types 1, 2, and 3

The safety of mass vaccination is proved in our material by the absence of any local and general reactions in the vaccinated who had no detectable poliovirus before vaccination

There was no evidence of association with live virus vaccination in separate sporadic poliomyelitis cases, notified in areas of large-scale live virus vaccinations

Convincing proofs are obtained of high immunologic and epidemiologic effectiveness of oral immunization with Sabin's vaccine strains using different schedules.*

According to our preliminary data, the use of trivalent mixture of vaccine strains Types 1, 2, and 3 proved to be highly effective: the greatest majority of children without prevaccination antibody developed antibody to the three types of poliovirus one month after oral vaccination with trivalent mixture

According to preliminary data, interference between the vaccine and latent-occurring enteroviruses, including Type 2 poliovirus, had no significant effect on the ultimate result of vaccination in that maximum completeness of immunization was achieved

Mass vaccination with live virus vaccine, which includes up to the present 6,250,000 persons, was not accompanied by any accidents, and went very smoothly and safely.

It may be assumed that oral immunization with live virus vaccine will allow in the nearest future to eradicate the menace of poliomyelitis epidemics Live virus vaccine from Sabin's strains has been approbated in our country as a safe and immunologically effective prophylactic preparation

* In Esthonia and Lithuania there was no unusual summer rise in the poliomyelitis incidence during June, July, and August 1959

14. FIELD TRIAL WITH SABIN'S LIVE POLIOVIRUS VACCINE IN CZECHOSLOVAKIA 1958-1959

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Dr Skovránek *(presenting the paper)* Introduction. Position prior to the vaccination with Sabin's vaccine. Czechoslovakia is one of the European countries where great attention is paid to the fight against poliomyelitis. In addition to special curative and rehabilitation centers, a special central virological department for research on poliomyelitis and the production of polio vaccine was built also in the Institute for Sera and Vaccines in Prague, as well as a number of regional virological laboratories attached to the Hygiene and Epidemiological Service Thus the prerequisites were created for comprehensive research on poliomyelitis—clinical, virological and epidemiological A dense net work of pediatric services in towns as well as rural districts which, apart from its typical preventive duties in the field of pediatrics, ensures also all vaccinations against infectious diseases during childhood, made it possible to carry out in the spring of 1957, the period of a threatening epidemic of polio, the first mass vaccination against polio with inactivated Salk vaccine Two doses were administered within the very short period of two months to two and a quarter million children, i e cca 87 per cent of the most susceptible children (18 per cent of the entire population) In 1958 a third injection of Salk's vaccine was administered to 2,181,529 children (by 31 July 1958) and the vaccination was systematically carried on also by vaccinating all children after they reached the age of seven months.

Some problems, of course, remained open; particularly the problem of the persistence of immunity after the administration of the inactivated vaccine After very encouraging experimental results with live attenuated polio viruses in the winter of 1958-59, we initiated in Czechoslovakia—with regard to the recommendations of the WHO Expert Committee (Geneva 1957)—one of the most extensive field trials of mass vaccination with the live polio vaccine

Before proceeding with a preliminary evaluation of this vaccination campaign, I shall give some basic data regarding the epidemiology of of poliomyelitis in Czechoslovakia.

Characteristic data on the morbidity, mortality, and lethality from polio in Czechoslovakia, on the state of seroimmunity of the population and child communities, the influence of vaccination with Salk's vaccine on the morbidity from polio and its clinical course were published in the *Journal of Hygiene, Epidemiology, Microbiology and Immunology* [1, 2] (in English)

I shall briefly recapitulate the most important of these data

1 *Morbidity, seasonal incidence, mortality, etc, of polio*

Since the first large epidemic of polio in the western part of Czechoslovakia in 1939 (with a mortality of 20 6 per 100,000 inhabitants), periodically, usually after five-year intervals, epidemics occur with a national morbidity of cca 20 per 100,000 inhabitants During the periods between epidemics, morbidity varies between 3 2 to 8 5 per 100,000 inhabitants a year. In both instances there are marked annual differences among individual regions and districts

The epidemic curves have a typical annual seasonal character with a maximum between July and September

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The mortality and lethality varies in relation to the morbidity rate as indicated below

MORBIDITY, MORTALITY AND LETHALITY OF POLIO IN CZECHOSLOVAKIA BETWEEN 1930 AND 1958

YEARS	MORBIDITY	MORTALITY	LETHALITY
1930—33	1 52	0 30	19 7
1934—37	1.25	0 29	23 2
1938—41	6 83	0 58	8 3
1942—45	5 83	0 69	11 8
1946—49	9 38	0 63	6 8
1950—53	10 03	0 73	7 3
1954—57	5 17	0 28	6 2
1958	2 41	0 12	4 8

Age incidence Since 1939 a gradual shift from the youngest age groups to older ones can be noted, whereby differences between the western and eastern part of the Republic persist, i e the shift of the morbidity to higher age groups in the western part of the country is more marked

According to *investigations of the seroimmunity*, made by Zácek *et al*[2] in three western regions of Czechoslovakia, and by Pesek[4] in the eastern part of Czechoslovakia, the investigated regions could be classified, according to the recommendations of the WHO, into groups II and III, respectively.

As far as the *distribution of polio viruses* in the territory of Czechoslovakia during recent years is concerned, it must be pointed out that in 1957 a great dispersal of Type 1 of the polio virus occurred (cca 97 per cent of some 400 strains isolated in Czechoslovakia in 1957 were identified as polio Type 1 strains; the remainder were strains of Type 3 polio virus; no Type 2 viruses were found). In 1958 a relatively extensive dispersal of Type 2 polio virus was recorded.

2. *The first vaccination campaign in 1957 using Salk's vaccine, and its results*

In the spring (May and June), at the onset of the epidemic, cca two and a quarter million children (2,246,811) were vaccinated with two doses of Salk's vaccine The majority of the children were aged 1-7 years, some of them 8-14 years The vaccination was carried out by the *intradermal route*, an average dose of 0 25 ml being used In autumn the vaccination was further extended, particularly in the group of children aged 8-14 years and in the spring of 1958 a third dose of vaccine was administered to the children who had received the first two injections in the spring of 1957

The vaccination status with Salk's vaccine in Czechoslovakia to 31 July 1958 is summarized thus

NUMBER OF CHILDREN VACCINATED AGAINST POLIOMYELITIS WITH SALK'S VACCINE
(Status on 31 July 1958)

AGE GROUPS	INJECTION	CZECH REGIONS		SLOVAKIA		CZECHOSLOVAKIA	
		No	% POPULATION	No	% POPULATION	No	% POPULATION
Pre-school age	1	823,371	93 3	447,485	90 4	1,270,856	92 3
School age (6–14 yrs)		1,069,297	77 6	456,478	78 2	1 525,775	77 8
Pre-school age	2	808,458	91 6	425,203	85 9	1,233,661	89 6
School age		1,052,698	76 4	435,267	74 6	1,487,965	75 9
Pre-school age	3	758,485	86 0	379,386	76 7	1,137,871	82 6
School age		747,785	54 3	295,873	50 7	1,043,658	53 2

Note

The figures for vaccinated pre-school children do not include children born in 1957 and 1958 who are vaccinated as soon as they reach the appropriate age; the number of these children is cca 20,000 per month

As far as the vaccination of children who have received the third injection is concerned, particularly school children, it is actually much higher because during the period between 1 August 1958 and 31 March 1959, more than half a million of these children had received their third injection.

The total number of children vaccinated with Salk's vaccine is at present cca 3 million children (22 per cent of the entire population)

From the results of the first vaccination campaign, using Salk's vaccine, discussed in greater detail by Skovránek *et al*,[7] we may conclude that the vaccination in 1957 had a favorable effect on the *morbidity*, particularly in the group of children aged 1-7 years, i e homogeneously vaccinated children, on the *age incidence*, where a flattening of the curve at the point of the typical maximum in the youngest children occurred, and on the *seasonal* incidence where a marked deformation of the seasonal epidemic curve in vaccinated children was recorded

In 1958 the favorable development of the morbidity from polio persisted. The national morbidity figures for paralytic polio were 2.4 per 100,000 inhabitants, however, with substantial variations in some regions (between a minimum of 0 5 and a maximum of 7 8 per 100,000 inhabitants), a phenomenon commonly observed every year

Basic Data on the Field Trial with Sabin's Live Polio Virus Vaccine

Despite the favorable results of the vaccination with Salk's vaccine in 1957 and 1958, there remained in Czechoslovakia—similarly as elsewhere in the world—some unsolved problems, particularly the problem of the persistence of immunity after vaccination with the inactivated vaccine in general, and after the intradermal route of administration in particular.

To elucidate these problems we elaborated the following program: To test in a sufficiently extensive field trial the safety of Sabin's live polio virus vaccine and its influence on the immunity against polio in children previously vaccinated with Salk's vaccine and in the general population—as compared with the immunity (and later also the morbidity) in children to whom three and four doses of Salk's vaccine had been administered, and those who were not vaccinated at all

In view of the fairly favorable epidemiological position in 1958 (the annual morbidity rate in Czechoslovakia was only 2 4 per 100,000 inhabitants) it was possible to plan our research as an experimental investigation, where the problems of the actual epidemiological situation did not play a particularly important role

Plan of investigations (research)

In view of the large percentage of children vaccinated with Salk's vaccine, the experiment had to be planned as follows:

(1) Selection of a suitable area for the vaccination with live polio vaccine;

(2) Selection of a suitable area with approximately the same sociological conditions for the vaccination with a fourth dose of Salk's vaccine,

(3) Regard the remaining areas where the vaccination with Salk's vaccine was carried out in 1957-58 as comparable with the two abovementioned experimental groups, and

(4) Focus the experimental work particularly on problems of the safety of the vaccination with Sabin's vaccine, on investigations of changes of the seroimmunity, the propagation of attenuated viruses in the population, and on long-term investigations of the polio morbidity

The chief aim of our work was to obtain an answer to the following problems particularly In the first place the problem of the safety of vaccination with Sabin's live polio viruses Subsequently, the following questions

(1) Changes of the seroimmunity—as compared with the seroimmunity in 1957, i e. prior to the administration of Salk's vaccine and prior to the administration of Sabin's live polio vaccine—in vaccinated children and in the general population after the administration of attenuated virus strains, particularly in individuals lacking antibodies against the different types of viruses.

(2) What will be the propagation of attenuated viruses in the population of regions where the vaccination was carried out and in neighboring regions—evaluated by the results of periodic examinations of random samples of feces at certain time intervals

(3) What will be the propagation of attenuated viruses in selected families with many children where only a part of the family was vaccinated, with special regard to the previous state of immunity and the age of different members of the family

(4) What will be the propagation of attenuated Type 1 viruses and how will the seroimmunity change in a special community of mentally defective children, where a maximum mutual fecal contamination and presence of other enteroviruses could be expected.

(5) How will the vaccination with the live vaccine influence the polio morbidity and the incidence of other virus affections of the CNS

(6) How will the repeated administration of Sabin's vaccine influence the formation of antibodies, particularly in those individuals where the first dose did not stimulate the formation of antibodies

We are aware of the fact that many of these problems are of a long term nature In view of the fact that the vaccination with live polio vaccine in Czechoslovakia was initiated in December 1958, we are able so far to give only a preliminary and yet incomplete answer to some of these problems

Basic Data on the Technical Procedure of the Vaccination Campaign with the Live Polio Vaccine

1 *Selection of the experimental regions*

The following four regions in the western part of the country were selected for the vaccination with the live polio vaccine (Ústí, Liberec, Jihlava, Ostrava), including two industrial regions (Ustí, Ostrava), one mixed (Liberec) and one predominantly agricultural (see map of Czechoslovakia) *

Two of the regions, Ústí and Jihlava, were selected, moreover, because in 1957—before the vaccination with Salk's vaccine was initiated—an extensive investigation of the seroimmunity of the population aged 0 40 years was undertaken in these regions and therefore they were very suitable for investigations of the changed seroimmunity before and after the administration of Sabin's vaccine.

As a control region for the administration of a fourth dose of Salk's vaccine on a large scale, the region of Plzen (Pilsen) was selected, which sociologically includes both components: highly industrialized centers and typically rural districts

Basic data on the number of inhabitants, the population density, and polio morbidity in the regions under investigation are given in the following table:

2 *Vaccine*

For the vaccination we used the vaccine supplied in October 1958 by Dr Albert B Sabin, before administration, the vaccine was kept at −20°C in the Institute for Sera and Vaccines in Prague; shortly before the vaccination was started, each type of vaccine was diluted separately. The diluted vaccine was kept in ordinary refrigerators and was never used longer than for seven days (Usually it was used within five days after dilution)

The Ministry of Health prepared detailed instructions for the campaign, containing necessary data regarding the handling of the vaccine, the technique of vaccination, follow-up investigations of the children after the vaccination, etc. Epidemiologists and virologists for the regions investigated were instructed, moreover, on the withdrawal of specimens, this did not encounter any particular difficulties because these workers had extensive experience from the vaccination campaign with Salk's vaccine

Sabin's vaccine was administered in all four regions simultaneously, in the recommended order: Type 1, Type 3, Type 2, with an interval of four weeks between the individual types The vaccine was administered in a spoonful of syrup in an amount of about 100,000 TCD_{50} No other method of administration of the attenuated viruses nor of a combination of viruses was tested.

The first type of virus was administered in December 1958 (15-20 December), the third type in January 1959 (12-16 January); and the second type in February 1959 (9-13 February).

* See p 569

TABLE 1. BASIC DATA FROM THE FOUR INVESTIGATED REGIONS, AS COMPARED WITH OTHER REGIONS

Region	Number of Inhabitants on 1 July 1958	Average Density of Popul. per 1 km²	Number of Patients and Polio Morbidity (per 100,000 Inhab. p.a.)											
			1953		1954		1955		1956		1957		1958	
Usti*	686,505	166	484	73.1	66	10.0	4	0.6	37	5.5	13	6.3	19	2.8
Liberec	509,944	120	258	51.9	54	10.9	—	—	21	4.2	33	6.5	9	1.8
Jihlava	439,027	66	35	8.1	7	1.6	1	0.2	6	1.4	43	9.8	14	3.2
Ostrava	960,365	214	64	7.0	288	31.6	32	3.4	54	5.7	43	4.4	24	2.5
Total	2,604,841	133	841	33.6	415	16.6	37	1.5	118	4.6	162	6.2	66	2.5
Plzeň†	580,305	74	64	11.3	20	3.5	12	2.1	25	4.3	27	4.7	12	2.1
All Czech regions incl. investigated regions	9,572,760	122	1895	20.6	794	8.5	133	1.4	388	4.1	639	6.7	231	2.4

Note:
* Four regions where Sabin's vaccine was administered
† Region where a fourth dose of Salk's vaccine was administered on a mass scale

Table 2. Number of Children in the Four Regions Investigated, by Age Groups, and Number of Children Vaccinated with Three Doses of Salk's Vaccine and Subsequently with Sabin's Vaccine

Age Group	No of Children Registered*	Vaccinated 3x With Salk's Vaccine		No of Children Vaccinated With Sabin's						Percentage of Total No ***
				Type 1		Type 3		Type 2		
		No	%	No	%†	No	%**	No	%‡	
Years										
2—3	44,370	33,179	74.8	24,405	73.6	21,646	88.7	19,192	88.7	43.3
3—4	45,236	40,467	89.5	29,731	73.5	26,409	88.8	23,644	89.5	52.3
4—5	45,734	40,506	88.6	29,967	74.0	26,691	89.1	23,919	89.6	52.3
5—6	46,973	42,104	89.6	29,961	71.2	26,741	89.3	23,631	88.4	50.3
2—6 years total	182,313	156,256	85.7	114,064	73.0	101,487	89.0	90,386	89.1	49.6
6—8 years	100,119	86,310	86.2	29,313	34.0	25,803	88.0	24,124	93.5	24.1
2—8 years total	282,432	242,566	85.9	143,377	59.1	127,290	88.8	114,510	90.0	40.5

Note:
* Children born before 1 November 1956
† Percentage from number of children vaccinated 3x with Salk's vaccine
** Percentage from number of children who received Type 1.
‡ Percentage from number of children who received Type 3.
*** Percentage from total number of children registered, regardless of vaccination with Salk's vaccine
Number of children vaccinated with Type 2, who were also fed all three types.
Number and percentage of children vaccinated 3x with Salk's vaccine by 31 July 1958

TABLE 1. BASIC DATA FROM THE FOUR INVESTIGATED REGIONS, AS COMPARED WITH OTHER REGIONS

Region	Number of Inhabitants on 1 July 1958	Average Density of Popul Per 1 km²	Number of Patients and Polio Morbidity (Per 100,000 Inhab p a)											
			1953		1954		1955		1956		1957		1958	
Usti*	686,505	166	484	73 1	66	10 0	4	0 6	37	5 5	43	6 3	19	2 8
Laberce	509,944	120	258	51 9	54	10 9	—	—	21	4 2	33	6 5	9	1 8
Jihlava	439,027	66	35	8 1	7	1 6	1	0 2	6	1 4	43	9 8	14	3 2
Ostrava	969,365	214	64	7 0	288	31 0	32	3 4	54	5 7	43	4 4	24	2 5
Total	2,604,841	133	841	33 6	415	16 6	37	1 5	118	4 6	162	6 2	66	2 5
Plzen†	580,305	74	64	11 3	20	3 5	12	2 1	25	4 3	27	4 7	12	2 1
All Czech regions incl investigated regions	9,572,760	122	1895	20 6	791	8 5	133	1 4	388	4 1	639	6 7	231	2 4

Note
* Four regions where Sabin's vaccine was administered
† Region where a fourth dose of Salk's vaccine was administered on a mass scale

TABLE 4 POLIO MORBIDITY IN FOUR REGIONS WHERE SABIN'S LIVE VACCINE WAS USED, AS COMPARED WITH OTHER REGIONS—JANUARY-MAY, 1957-1958

		January—May		
		1957	1958	1959
Regions where Sabin's vaccine was administered (No of inhabitants on July 1, 1958—2,604,841)	Absolute number	49	11	5
	Morbidity per 100,000 inhabitants	1 88	0 42	0 19
Other (control) regions (No of inhabitants on July 1, 1958—6,967,919)	Absolute number	116	47	14
	Morbidity per 100,000 inhabitants	1 66	0 67	0 20
Czech regions total (No of inhabitants on July 1, 1958—9,572,760)	Absolute number	165	58	19
	Morbidity per 100,000 inhabitants	1 72	0 61	0 20

Note
None of the five cases of polio in the regions investigated Sabin's vaccine had been administered

and the development of the disease in a total of 143,377 children who had been fed Type 1 live polio virus vaccine in December 1958

Naturally we devoted particular attention to this case From the results of detailed investigations given in Table 6 we concluded that during the period of administration of attenuated virus Type 1, the patient became infected with Type 3 of the poliovirus, which interfered for a certain time with the administered Type 1 of the attenuated virus (The clinical data concerning this case are given in Appendix 1)

As a matter of interest, we should like to mention that during the period of vaccination with Salk's vaccine, which was carried out at the onset of the epidemic, we revealed several tens of similar cases of chronological coincidence (60 cases of polio within 30 days after the first dose of Salk's vaccine, 52 cases within 30 days after the second dose of Salk's vaccine)

To the problem of chronological coincidence of the vaccination and the possible development of the disease great attention will have to be paid and this possibility will have to be explained in advance to the lay public (including medical laymen), particularly in countries which are starting with the vaccination The number of possible cases of the disease which develop in some time relation with the vaccination shortly after the administration of the vaccine will depend on the actual epidemiological situation of the country or area where the vaccination with the live vaccine will be carried out

For the problem of safety of the vaccination with the live vaccine it is important *how many persons who received the live vaccine are lacking homologous antibodies*, this is particularly important in a country where the vaccination with the inactivated vaccine was carried out on such a large scale as was the case in Czechoslovakia This problem is discussed in the next part of our paper containing the results of virological and serological investigations From these it appears that cca 35 per cent of the children which were fed Type 1 of the attenuated virus strain lacked antibodies against Type 1 prior to its administration, and cca 44 per cent lacked antibodies against Type 3 (Expressed in the estimated number this represents about 48,000 children lacking antibodies against Type 1 and cca 55 000 against Type 3)

(More detailed data are given in a subsequent part of this paper)

Mainly children aged 2-6 years and some children aged 6-8 years who in the 1957-58 campaign had received three doses of Salk's vaccine (by the intradermal route) were vaccinated.

Before the administration of Sabin's vaccine the state of seroimmunity was investigated in two of the regions in November 1958 (Ústí, Jihlava) and previously in 1957. (The serological results as well as those obtained after the administration of Sabin's vaccine are given in the subsequent part of this paper.)

The vaccination was on a voluntary basis. The parents were informed of the vaccination by a health education campaign and by individual invitations containing instructions regarding the principles of the vaccination. (Example from the Jihlava region.) There was great interest in the vaccination among the population. The number of children who attended the vaccination was higher than the number of actually vaccinated children, as a certain percentage of the children was excluded owing to contraindications. The smaller number of children vaccinated with Types 3 and 2 was mainly due to the increased incidence of acute catarrhs of the upper respiratory routes during the winter period.

The number of vaccinated children is given in Table 2.

An example of the causes of why the vaccination was not carried out for one region is given in Table 3.

TABLE 3. EXAMPLE OF THE AVERAGE STRUCTURE OF THE CAUSES WHY VACCINATION WITH SABIN'S VACCINE WAS NOT CARRIED OUT (Ostrava Region)

	PERCENTAGE OF INVITED CHILDREN WHO DID NOT ATTEND	PERCENTAGE EXCLUDED BY DOCTOR, IN VIEW OF CONTRA-INDICATIONS
TYPE 1	13.3	13.6
TYPE 3	6.4*	5.5
TYPE 2	2.9†	5.5

Note:
* Percentage of the number of children who were vaccinated with Type 1.
† Percentage of the number of children who were vaccinated with Type 1 and Type 3.

The vaccination was carried out by vaccination teams of the ordinary health services (always headed by a pediatrician). The vaccine was administered by a nurse to children examined before the vaccination by the doctor. The number of children vaccinated per hour was 60-80, depending on the number of doctors. The organization and supervision of the campaign was the responsibility of epidemiologists of the Hygiene and Epidemiological Service. A total of 250 doctors and 420 nurses took part in the vaccination.

Safety of the Vaccine

When evaluating the problem of safety of the vaccination with Sabin's vaccine, we had to rely on two sources of information:

(1) Reports from the vaccinating doctors on side-effects recorded during and after the vaccination; and

(2) Reports on the incidence of polio in the regions investigated, as compared with other regions.

From the reports of vaccinating doctors who did not reveal any reactions which aroused the attention of parents or health workers, we could conclude that *the administration of all three types of Sabin's live polio virus vaccine is quite safe.*

A similar picture is obtained from the results of studies on the incidence of polio in the investigated regions as compared with the other regions, which are listed in Table 4, which summarizes the data from all four regions during the first five months of 1959, compared with the same period in the previous two years.

This table only illustrates that, as compared with other regions, no increase in the polio incidence occurred in the investigated regions (so far during the five months of the investigation).

In none of the five cases of polio reported, which developed during the first months of 1959 in the regions investigated, Sabin's vaccine had been administered.

A more detailed analysis of the five cases mentioned above is provided by the results summarized in Table 5.

Thanks to the favorable season of the year with a minimal natural incidence of polio, we encountered only one case of *chronological coincidence* of the administration of attenuated viruses

TABLE 6

Vysinová Jaroslava, 4 years
Clinical diagnosis Paralysis of the right lower leg
Beginning of illness 28 Dec 1958
Date of hospitalization 3 Jan 1959

			Antibody Levels of Sera Taken January, 1959					
			5th	7th	13th	16th	19th	28th
POLIO (Antibodies)	pH test	1	<8	<8	2048	512	64	128
		2	64	32	128	32	64	32
		3	64	256	128	32	64	32
	cytopath test	1	<8	<8	64	16	64	4
		2	<8	<8	8	8	16	4
		3	64	256	128	32	64	32
	Complement fixation (avidity)	1	0	—*	—	—	—	23
		2	0	—	—	—	—	11
		3	0	—	—	—	—	9
	Isolation exp (stool)		neg	neg	Type 1 10^{4} (gr)	Type 1 10^{4} (gr)	—	neg
ECHO 9	pH test		<32	512	256	64	256	16
	CP test		<32	256	512	32	512	32
COXSACKIE A9	CP test		<32	8	32	32	128	1024
COXSACKIE B3	pH test		<32	64	128	32	<8	16
	CP test		<8	<8	<8	<8	<8	<8

* Not done

TABLE 5. MORE DETAILED DATA ON THE POLIO CASES REPORTED FROM JANUARY TO MAY 1959 IN THE INVESTIGATED REGIONS

Region	No. of Cases	Age	Vaccinated Salk	Vaccinated Sabin	Was Live Vaccine Administered in the Family?	Beginning of the Illness	Virus Isolation	Virological Findings: Serology‡ Type	CF Titer	CPE	CF Avidity	Clinical Picture
Usti	2	7 months	no	no	no	25 Feb	negative	not examined				paraparesis of lower limbs
		5 years	3x	no	no	22 Jan	negative	not examined				paresis of the right lower limb
Liberec	2*	11 years	3x	no	yes	22 Apr	negative	1	4/8	32/16	0/4	paresis absent (spasm of back muscles)
								2	4/8	8/16	1/5	
								3	0/4	32/16	0/1	
		36 years†	no	no	yes†	4 Feb	negative	1	4/4	4/4	0/1	paraparesis of lower limbs
								2	4/32	128/512	1/19	
								3	0/0	4/4	0/0	
Jihlava	1	4 years	1x	no	no	18 Apr	polio Type 2	1	8/8	not exam	0/0	paraparesis of lower limbs
								2	32/32	not exam	26/30	
								3	32/32	not exam	32/35	
Ostrava	—	—	—	—	—	—	—	—	—	—	—	—

Notes:
* As a result of clinical and serological findings, the first case can be excluded from the series.
† During time of vaccination, did not live with the family; returned 3 days before the disease developed
‡ In serological results numerator=acute serum specimen, denominator=convalescent blood

TABLE 6

Vysinová Jaroslava, 4 years
Clinical diagnosis: Paralysis of the right lower leg
Beginning of illness: 28 Dec 1958
Date of hospitalization: 3 Jan 1959

			Antibody Level of Sera Taken January, 1959					
			5th	7th	13th	16th	19th	28th
POLIO (Antibodies)	pH test	1	<8	<8	2048	512	64	128
		2	64	32	128	32	64	32
		3	64	256	128	32	64	32
	cytopath. test	1	<8	<8	64	16	64	4
		2	<8	<8	8	8	16	4
		3	64	256	128	32	64	32
	Complement fixation (avidity)	1	0	—*	—	—	—	23
		2	0	—	—	—	—	11
		3	0	—	—	—	—	9
	Isolation exp. (stool)		neg	neg	Type I 10[illegible] (gr.)	Type I 10[illegible] (gr.)	—	neg
ECHO 9	pH test		<32	512	256	64	256	16
	CP test		<32	256	512	32	512	32
COXSACKIE A9	CP test		<32	8	32	32	128	1024
COXSACKIE B3	pH test		<32	64	128	32	<8	16
	CP test		<8	<8	<8	<8	<8	<8

* Not done.

VIROLOGICAL AND SEROLOGICAL INVESTIGATIONS

METHODS

Isolation of viruses

Specimens of feces were kept before examination at −20°C In Earl's solution with 0 5% lactalbumin hydrolysate 20% suspensions were prepared (the stools were weighed) and their pH was adjusted by means of a bicarbonate solution to pH 7 5 or a higher pH The 20% suspensions were centrifuged for 60 minutes at 4,500 5,000 r p m and to the supernatant 1,000 units of penicillin and streptomycin per ml were added

The isolation was carried out by inoculating 0 1 ml of the 20% suspension into three tissue cultures from versenated monkey kidney cells, after two hours in the rollers at 35-36°C, the infected medium was discarded and replaced by a new medium containing 0 0015 g of sodium bicarbonate per ml in Earl's solution with 0.5% of lactalbumin hydrolysate without serum Next the medium was changed on the fourth day after the beginning of the experiment and the cultures were investigated daily for eight days If a non-specific degeneration of the tissue culture occurred, blind passages were performed

If the isolation experiment was positive, the identification and titration of the viruses were repeated with the original suspensions of feces, diluted 1/20 (1% suspension) For the titration always the two test tube cultures were inoculated with 0 1 ml of 10^{-1} - 10^{-6} of diluted suspensions of feces If the titer was lower than $10^{2.5}$ per g of feces, the identification experiments were repeated with the infected tissue fluid

Evidence of neutralizing antibodies

After centrifuging the blood the sera were inactivated for 20 minutes at 60°C and after the addition of 500 units of penicillin and streptomycin per ml they were kept at −20°C The incubation period of the serum with the virus in all experiments was 1 hour at room temperature.

The color tests were carried out in polystyrene panels; after incubation of 0.2 ml. of the appropriately diluted serum with 0.2 ml of the medium containing approximately 100 TCD_{50} of the virus under a layer of paraffin oil, to every cup were added 0 2 ml. of the suspension of versenated monkey cells, numbering 25,000 cells per cup As a medium we used solution 199 with 5% of monkey serum which did not contain any inhibitors against any of the three types of polio viruses. The readings were taken on the 5th or 6th day of the experiment

The neutralizing antibodies were investigated also by means of the cytopathogenic test in test tube cultures prepared from versenated monkey kidney cells The mixture of serum (0 05 ml of the total amount of serum) and 100 TCD_{50} of the virus were incubated for one hour at room temperature and from each dilution of serum one test tube culture was inoculated. As a medium we used Earl's solution with 0 5% of lactalbumin hydrolysate with maintenance 2% of horse serum Readings were taken on the 5th day of the experiment.

For all neutralization reactions we used the strains Brunhilde, MEF-1 and Sauckett, and the sera were examined in groups of about 300 sera, selected in such a manner that one series of experiments included approximately the same number of samples from both collections of sera and from all age groups

MATERIAL

1 Serological investigations of the population

When assembling the material for our serological surveys of the general population, we proceeded similarly as in the first serological survey undertaken in Czechoslovakia in 1957

Between January and March 1957 from three Czech regions (Ústí, Jihlava, Praha) more than 3,000 samples of blood were obtained In November 1958 within three weeks in two regions (Ústi and Jihlava) more than 1,200 samples were collected in each region and in the course of three weeks in April 1959 another 1,200 samples were obtained in the above two regions, i e, a total of 5,000 blood samples in 1958 and 1959

As has been mentioned in the introduction, the Ústí and Jihlava regions were selected for the serological investigations in 1958 and 1959 because in both these regions the first serological surveys had already been carried out in 1957 (before the vaccination with Salk's vaccine).

Blood samples were collected in both regions at random from all parts of the region in pro-

portion to the number of inhabitants, and in no instance were samples from the same individuals taken in November 1958 and April 1959.

Samples were withdrawn from individuals of different ages, divided into thirteen groups:

group.			
1	0-5 months	number of samples:	60
2	5-11 months	"	60
3	1-2 years	"	60
4	2-3 "	"	60
5	3-4 "	"	60
6	4-5 "	"	60
7	5-6 "	"	60
8	6-10 "	"	120
9	10-14 "	"	120
10	15-19 "	"	120
11	20-29 "	"	120
12	30-39 "	"	120
13	40+ "	"	120

The blood samples were taken mostly from patients hospitalized (during the first few days after the disease had begun) at different departments of public hospitals in the region, with the exception of persons suffering from infectious diseases and those who had in their case history an infectious disease of the CNS Where it did not prove possible to obtain a sufficient number of blood samples in the hospitals, the samples were taken in crèches, nurseries and schools From any one community, however, never more samples were taken than one seventh of the total number of members of the community The samples were taken from children below 6 years only and did not exceed 10% of the total material

All blood samples were taken by two teams of doctors and nurses participating in the campaign, who travelled to the respective areas (In 1958 and 1959 they covered more than 50,000 km) The blood samples were always taken into syringes, after the withdrawal, they were kept on ice and, within 48 hours at the latest, the serum was separated and inactivated and 500 units of penicillin and streptomycin per ml were added to it and it was kept frozen before the examination

The blood samples obtained in the Usti region were examined quantitatively between 10 April and 15 May 1959 by means of the pH test in four dilutions and qualitatively in one dilution 0 05 ml of the total amount of serum as well as by the CPE test for the content of neutralizing antibodies against all types of polioviruses

2. Serological investigations of paired sera

We succeeded in obtaining these from more than 400 individuals of different age groups from whom blood samples were taken during the first collection in the Ústí and Jihlava region in November 1958 (one month before the first dose of live polio vaccine was administered in the region) and the second one, in April 1959, i e, cca 4 3 and 2 months after the vaccination with the live polio vaccine These paired sera form a separate group which was not included in the material of the first group (serological surveys in the general population). In all individuals of this group we investigated carefully who was and who was not vaccinated with all types of attenuated polio viruses, and 96 pairs of sera obtained from vaccinated children aged 0 8 years and 258 pairs of sera obtained from persons of different age groups which were not vaccinated with Sabin's vaccine were examined between 15 April and 10 May Five dilutions of serum (1/4—1/1,024) were used for testing the presence of neutralizing antibodies against three types of polioviruses by the pH test as well as the CPE test tube test Approximately 80% of the paired sera were examined also by the two-dimensional test described by Black and Melnick for complement-fixing antibodies against all types of polioviruses.

3 Family studies

At the beginning of 1959 it proved possible to gain the cooperation of more than 25 families in Czechoslovakia Twenty-two of these families could be included in the results of the experiments (13 families in Prague, 6 in the Gottwaldov region, and three in the region of České Budejovice), all from areas where the live polio vaccine was not used for the vaccination of children

For these investigations large families were selected with at least four children, in a number of these families there were however 6-8 children, and in some as many as ten The total number of individuals investigated was 161 Before this investigation was begun, blood samples were taken from all members of the families, one portion was examined immediately and neutralizing antibodies were estimated quantitatively by means of the pH test, and the second portion of the sera was kept in a frozen state Simultaneously with the withdrawal of the first blood samples, samples of feces were collected also and

VIROLOGICAL AND SEROLOGICAL INVESTIGATIONS

METHODS

Isolation of viruses

Specimens of feces were kept before examination at −20°C. In Earl's solution with 0.5% lactalbumin hydrolysate 20% suspensions were prepared (the stools were weighed) and their pH was adjusted by means of a bicarbonate solution to pH 7.5 or a higher pH. The 20% suspensions were centrifuged for 60 minutes at 4,500-5,000 r.p.m. and to the supernatant 1,000 units of penicillin and streptomycin per ml were added.

The isolation was carried out by inoculating 0.1 ml. of the 20% suspension into three tissue cultures from versenated monkey kidney cells, after two hours in the rollers at 35-36°C, the infected medium was discarded and replaced by a new medium containing 0.0015 g. of sodium bicarbonate per ml. in Earl's solution with 0.5% of lactalbumin hydrolysate without serum. Next the medium was changed on the fourth day after the beginning of the experiment and the cultures were investigated daily for eight days. If a non-specific degeneration of the tissue culture occurred, blind passages were performed.

If the isolation experiment was positive, the identification and titration of the viruses were repeated with the original suspensions of feces, diluted 1:20 (1% suspension). For the titration always the two test tube cultures were inoculated with 0.1 ml. of 10^{-1}-10^{-6} of diluted suspensions of feces. If the titer was lower than $10^{-2.5}$ per g. of feces, the identification experiments were repeated with the infected tissue fluid.

Evidence of neutralizing antibodies

After centrifuging the blood, the sera were inactivated for 20 minutes at 60°C. and after the addition of 500 units of penicillin and streptomycin per ml. they were kept at −20°C. The incubation period of the serum with the virus in all experiments was 1 hour at room temperature.

The color tests were carried out in polystyrene panels; after incubation of 0.2 ml. of the appropriately diluted serum with 0.2 ml. of the medium containing approximately 100 TCD_{50} of the virus under a layer of paraffin oil, to every cup were added 0.2 ml. of the suspension of versenated monkey cells, numbering 25,000 cells per cup. As a medium we used solution 199 with 5% of monkey serum which did not contain any inhibitors against any of the three types of polio viruses. The readings were taken on the 5th or 6th day of the experiment.

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As has been mentioned in the introduction the Ústí and Jihlava regions were selected for the serological investigations in 1958 and 1959 because in both these regions the first serological surveys had already been carried out in 1957 (before the vaccination with Salk's vaccine).

Blood samples were collected in both regions at random from all parts of the region in pro-

tions; the samples were taken proportionally in the entire region according to population density. In one campaign some 120-150 and sometimes more samples were collected in every region. The feces were collected mostly from children under 10 years of age but also from some adults living in child communities (crèches, nursery school, schools, hostels) in such a way that in one community not more than samples from two persons were taken. If the same virus was isolated from two individuals living in the same community, only the results of one isolation experiment (1 strain) were included into the evaluation. During the subsequent collection, samples of feces were obtained in the same region in a similar manner, from individuals living in other communities.

The first series of samples of feces in all five regions was collected at the end of November and the beginning of December 1958, and in no instance later than 15 December, when the mass vaccination of children with Type 1 of the live polio vaccine was started in Czechoslovakia. As can be seen from the tables presented on the results, further collections of material were organized in the regions in a similar manner, at different intervals, after the beginning of the field trial with the live vaccine. Three more series of samples were collected so far (in January, April, and May 1959) and the results of isolation experiments from collections one, two, and three are available.

To obtain more accurate information on the incidence of enteroviruses in the population of the Czech regions during the period before the *onset of* the epidemic season, the collection of samples of feces was extended at the beginning of June 1959, and between 1-15 June samples will be collected from practically the entire territory of the Republic in a similar manner as previously but including a greater proportion of children below the age of six years.

Results of Serological and Virological Investigations

1. *Results of serological investigations in the general population*

The results summarizing the percentage incidence of neutralizing antibodies in the population in one of the investigated regions in Czechoslovakia (Ústí nad Labem) in 1957, i.e., before the mass vaccination with Salk's vaccine (estimated by the CPE test), in November 1958 (one month before the administration of Sabin's vaccine and in 1959, i.e., two months after the administration of the last dose of live vaccine (estimated by the CPE and pH test) are given in Table 7 for Type 1, Table 8 for Type 2, and Table 9 for Type 3. (In the chapter on *Material* it has been mentioned already that all sera were obtained by random selection and were in no instance taken from the same individuals.)

Though we are at present concentrating our attention on problems related to the influence of vaccination with live poliovaccine on the serological immunity of the *population, it appears* from a comparison of the results obtained in 1957 (sera taken during the first three months of 1957) and in 1958 (sera taken in November 1958) that in corresponding age groups a certain increase of the antibodies against Type 1 during the 20-month period between the sampling in 1957 and 1958 can be *observed. This increase* was not observed in Type 3, while there was a significant increase of antibodies against Type 2 in the period investigated.

Apart from the possible effect of mass immunization of the child population with Salk's vaccine, particularly as far as antibodies against Type 2 are concerned, the most *probable possible* explanation must be sought in the fact that in the course of 1957 *in the Czechoslovak areas* under observation a high spread of polioviruses Type 1 *occurred (from a total of cca 400 strains* isolated mostly from sick individuals, 96.3% were identified as Type 1, 3.3% as Type 3, and not a single strain as Type 2) and in 1958 a relatively substantial increase of Type 2 *strains* (from a total of cca 200 strains isolated from *patients* 15 Type 2 strains were revealed, the remaining strains being Type 1 and *not a single* strain of Type 3 was isolated) Table 10.

A comparison of the state of seroimmunity of *the general* population in the Ústí region before and after the administration of the live polio *vaccine to children aged* 2-8 years reveals a significant increase—on an average a 20-30% increment of antibodies against Types 1 and 3, *particularly in those* age groups which were vaccinated with the live vaccine. *On the other hand,*

these were subsequently collected at regular weekly intervals throughout the entire campaign, i e, more than 15 weeks, from all members of the family. A total of 2,500 samples of feces were obtained in the families and examined for the presence of enteroviruses The samples were kept by the experimental persons in a cold place (samples were collected mostly in winter) and collected on the same day by the laboratory workers and transported on ice to the laboratory where they were kept at −20°C

Approximately 30 days after the administration of the last dose of the attenuated virus (Type 2), a second series of blood samples was taken from all 161 individuals and these were examined during the period between 20 April and 10 May 1959 in five serum dilutions (1/4 to 1/1,024) along with the first samples of sera, by means of the pH test and CPE test for the presence of neutralizing antibodies against all types of polioviruses

The vaccination with the attenuated polioviruses was carried out as follows: in every family cca half the members were vaccinated, i e, one child was fed and another child of similar age was not fed the virus. In a similar way the adults were vaccinated also, usually the mothers only.

Type 1 of the attenuated virus was in most instances administered in January 1959, the same individuals received Type 3 after five weeks, and after another five weeks, Type 2 of the attenuated virus The vaccination was carried out by doctors, every child was first given some biscuits and subsequently 100,000 TCD_{50} of the virus in the form of two drops of 1 10 diluted original Sabin's vaccine in a spoonful of diluted syrup

4 Investigations in the Institute for Mentally Defective Children (Oparany)

In the Institute for Mentally Defective Children in the locality of Oparany (region Ceské Budejovice) where the population had not been vaccinated with live polio vaccine, there are some 400 children aged 4-16 years, most of whom live in the institute permanently Most of these children live in two buildings (house A and B) situated at a distance of cca 60 meters and neither inmates nor the staff communicate. Both buildings, however, obtain their meals from the same kitchen, have a common laundry, and both buildings are visited regularly by two senior physicians

In the Oparany institute a total of 78 children were selected for the investigation, 53 of whom live in building A, and 24 in building B. From all the investigated children and from most of the adult staff the first blood samples and samples of feces were taken on 15 December 1958 and during the third week in December 17 children of different age groups in building A were vaccinated with 100,000 TCD_{50} of the LSc strain of the attenuated polio virus. After the first collection of samples of feces, further samples were collected at regular weekly intervals from all the children investigated and from the adult staff—so far for 23 weeks A total of more than 2,000 samples of feces were collected In the course of the investigation, control blood samples were taken from some of the children and in April 1959 second or further samples of blood were taken from all the persons under investigation The first samples were examined quantitatively by means of the pH test immediately after the samples had been withdrawn, and the remaining samples were examined subsequently The virological examination of the Oparany community is still in progress and the material will be collected for several more months

5 Investigations of the spread of enteroviruses among the population of different regions in Czechoslovakia

Before 15 December 1958, i e, before the field trial with the live polio vaccine, an extensive investigation was started of the spread of enteroviruses in the population of two regions where the live vaccine was used (Liberec and Jihlava), and in the population of three neighboring control regions (Hradec Králové, Gottwaldov and Ceské Budejovice) where the live polio vaccine was not administered to the children For this purpose, samples of feces were collected in all five regions and were examined for the presence of enteroviruses in the virological laboratories of the regional hygiene and epidemiological stations on tissue cultures from versenated monkey cells, supplied to these laboratories by the Central Institute for Sera and Vaccine in Prague Thus, the entire material in Czechoslovakia was examined on the same tissue cultures

The collection of samples of feces in all regions proceeded according to the following instruc-

tions the samples were taken proportionally in the entire region according to population density. In one campaign some 120-150 and sometimes more samples were collected in every region. The feces were collected mostly from children under 10 years of age but also from some adults living in child communities (crèches, nursery school, schools, hostels) in such a way that in one community not more than samples from two persons were taken. If the same virus was isolated from two individuals living in the same community, only the results of one isolation experiment (1 strain) were included into the evaluation. During the subsequent collection, samples of feces were obtained in the same region in a similar manner, from individuals living in other communities.

The first series of samples of feces in all five regions was collected at the end of November and the beginning of December 1958, and in no instance later than 15 December, when the mass vaccination of children with Type 1 of the live polio vaccine was started in Czechoslovakia. As can be seen from the tables presented on the results, further collections of material were organized in the regions in a similar manner, at different intervals, after the beginning of the field trial with the live vaccine. Three more series of samples were collected so far (in January, April, and May 1959) and the results of isolation experiments from collections one, two, and three are available.

To obtain more accurate information on the incidence of enteroviruses in the population of the Czech regions during the period before the onset of the epidemic season, the collection of samples of feces was extended at the beginning of June 1959, and between 1-15 June samples will be collected from practically the entire territory of the Republic in a similar manner as previously, but including a greater proportion of children below the age of six years.

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The results summarizing the percentage incidence of neutralizing antibodies in the population in one of the investigated regions in Czechoslovakia (Ústí nad Labem) in 1957, i.e., before the mass vaccination with Salk's vaccine (estimated by the CPE test), in November 1958 (one month before the administration of Sabin's vaccine and in 1959, i.e., two months after the administration of the last dose of live vaccine (estimated by the CPE and pH test) are given in Table 7 for Type 1, Table 8 for Type 2, and Table 9 for Type 3. (In the chapter on Material it has been mentioned already that all sera were obtained by random selection and were in no instance taken from the same individuals.)

Though we are at present concentrating our attention on problems related to the influence of vaccination with live poliovaccine on the serological immunity of the population, it appears from a comparison of the results obtained in 1957 (sera taken during the first three months of 1957) and in 1958 (sera taken in November 1958) that in corresponding age groups a certain increase of the antibodies against Type 1 during the 20-month period between the sampling in 1957 and 1958 can be observed. This increase was not observed in Type 3, while there was a significant increase of antibodies against Type 2 in the period investigated.

Apart from the possible effect of mass immunization of the child population with Salk's vaccine, particularly as far as antibodies against Type 2 are concerned the most probable possible explanation must be sought in the fact that in the course of 1957 in the Czechoslovak areas under observation a high spread of polioviruses Type 1 occurred (from a total of cca 400 strains, isolated mostly from sick individuals, 96.3% were identified as Type 1, 3.3% as Type 3, and not a single strain as Type 2) and in 1958 a relatively substantial increase of Type 2 strains (from a total of cca 200 strains isolated from patients 15 Type 2 strains were revealed, the remaining strains being Type 1 and not a single strain of Type 3 was isolated). Table 10.

A comparison of the state of seroimmunity of the general population in the Ústí region before and after the administration of the live polio vaccine to children aged 2-8 years reveals a significant increase—on an average a 20-30% increment of antibodies against Types 1 and 3, particularly in those age groups which were vaccinated with the live vaccine. On the other hand,

these were subsequently collected at regular weekly intervals throughout the entire campaign, i.e., more than 15 weeks, from all members of the family. A total of 2,500 samples of feces were obtained in the families and examined for the presence of enteroviruses. The samples were kept by the experimental persons in a cold place (samples were collected mostly in winter) and collected on the same day by the laboratory workers and transported on ice to the laboratory where they were kept at −20°C.

Approximately 30 days after the administration of the last dose of the attenuated virus (Type 2), a second series of blood samples was taken from all 161 individuals and these were examined during the period between 20 April and 10 May 1959 in five serum dilutions (1:4 to 1:1,024) along with the first samples of sera, by means of the pH test and CPE test for the presence of neutralizing antibodies against all types of polioviruses.

The vaccination with the attenuated polioviruses was carried out as follows: in every family cca half the members were vaccinated, i.e., one child was fed and another child of similar age was not fed the virus. In a similar way the adults were vaccinated also, usually the mothers only.

Type 1 of the attenuated virus was in most instances administered in January 1959, the same individuals received Type 3 after five weeks, and after another five weeks, Type 2 of the attenuated virus. The vaccination was carried out by doctors, every child was first given some biscuits and subsequently 100,000 TCD_{50} of the virus in the form of two drops of 1:10 diluted original Sabin's vaccine in a spoonful of diluted syrup.

4. Investigations in the Institute for Mentally Defective Children (Oparany)

In the Institute for Mentally Defective Children in the locality of Oparany (region České Budejovice) where the population had not been vaccinated with live polio vaccine, there are some 400 children aged 4-16 years, most of whom live in the institute permanently. Most of these children live in two buildings (house A and B) situated at a distance of cca 60 meters and neither inmates nor the staff communicate. Both buildings, however, obtain their meals from the same kitchen, have a common laundry, and both buildings are visited regularly by two senior physicians.

In the Oparany institute a total of 78 children were selected for the investigation, 53 of whom live in building A, and 24 in building B. From all the investigated children and from most of the adult staff the first blood samples and samples of feces were taken on 15 December 1958 and during the third week in December 17 children of different age groups in building A were vaccinated with 100,000 TCD_{50} of the LSc strain of the attenuated polio virus. After the first collection of samples of feces, further samples were collected at regular weekly intervals from all the children investigated and from the adult staff—so far for 23 weeks. A total of more than 2,000 samples of feces were collected. In the course of the investigation, control blood samples were taken from some of the children and in April 1959 second or further samples of blood were taken from all the persons under investigation. The first samples were examined quantitatively by means of the pH test immediately after the samples had been withdrawn, and the remaining samples were examined subsequently. The virological examination of the Oparany community is still in progress and the material will be collected for several more months.

5. Investigations of the spread of enteroviruses among the population of different regions in Czechoslovakia

Before 15 December 1958, i.e., before the field trial with the live polio vaccine, an extensive investigation was started of the spread of enteroviruses in the population of two regions where the live vaccine was used (Liberec and Jihlava), and in the population of three neighboring control regions (Hradec Králové, Gottwaldov and České Budejovice) where the live polio vaccine was not administered to the children. For this purpose, samples of feces were collected in all five regions and were examined for the presence of enteroviruses in the virological laboratories of the regional hygiene and epidemiological stations on tissue cultures from versenated monkey cells, supplied to these laboratories by the Central Institute for Sera and Vaccine in Prague. Thus, the entire material in Czechoslovakia was examined on the same tissue cultures.

The collection of samples of feces in all regions proceeded according to the following instruc-

TABLE 8 STATUS OF TYPE 2 ANTIBODY IN THE GENERAL POPULATION OF ÚSTÍ REGION BEFORE AND AFTER THE USE OF SALK VACCINE IN CHILDREN UP TO 14 YEARS OF AGE, AND LIVE VIRUS VACCINE (SABIN) IN CHILDREN 2 TO 8 YEARS OF AGE

	Before Salk Vaccine Early 1957		8 Months After the 3rd Dose of Salk Vaccine, November 1958		2 Months After Administration of Sabin Vaccine Type 2, April 1959	
Age Group Years	Number Tested	Per Cent Positive CPE Test	Number Tested	Per Cent Positive CPE / pH	Number Tested	Per Cent Positive CPE / pH
0—0.5	68	46.0	61	50.8 / 54.1	61	52.5 / 70.5
0.5—1	62	14.5	53	28.3 / 34.0	57	28.1 / 42.1
1—2	65	9.2	51	64.7 / 68.6	61	50.8 / 60.7
2—3	46	31.8	43	62.8 / 69.8	53	66.0 / 79.3
3—4	38	50.2	43	60.5 / 62.8	53	66.0 / 79.3
4—5	31	29.0	49	71.4 / 75.5	40	75.5 / 82.5
5—6	17	37.5	40	85.0 / 87.5	53	83.0 / 94.3
6—10	62	72.9	91	90.0 / 93.0	98	86.7 / 98.0
10—15	90	80.0	93	87.8 / 88.2	106	90.6 / 93.4
15—20	82	73.2	89	78.7 / 83.2	88	77.3 / 88.6
20—30	52	82.7	94	87.2 / 89.4	118	89.1 / 86.4
30—40	27	89.0	73	90.4 / 89.0	127	85.0 / 94.5
40+	(30+)		114	90.4 / 95.6	123	90.2 / 97.6
Total	638		894		1038	

strains were isolated in a relatively high number [illegible] in 1959 type 2

TABLE 7 STATUS OF TYPE 1 ANTIBODY IN THE GENERAL POPULATION OF THE ÚSTÍ REGION BEFORE AND AFTER THE USE OF SALK VACCINE IN CHILDREN UP TO 14 YEARS OF AGE, AND LIVE VIRUS VACCINE (SABIN) IN CHILDREN 2 TO 8 YEARS OF AGE

	Before Salk Vaccine Early 1957		8 Months After the 3rd Dose of Salk Vaccine, 1 Month Before the Sabin Vaccine, November 1958		4 Months After Administration of Sabin Vaccine Type 1 Live Virus, April 1959	
Age Group Years	Number Tested	Per Cent Positive CPE Test	Number Tested	Per Cent Positive CPE / pH	Number Tested	Per Cent Positive CPE / pH
0—0 5	66	33 3	61	34 4 / 47 5	61	36 6 / 52 5
0 5—1	62	0	53	15 1 / 13 2	57	19 3 / 22 8
1—2	65	21 5	51	33 3 / 41 7	61	23 0 / 39 3
2—3	46	44 8	43	37 2 / 48 8	53	60 4 / 64 2
3—4	38	42 1	43	41 9 / 44 2	53	69 8 / 73 6
4—5	31	61 3	49	55 1 / 63 3	40	67 5 / 75 5
5—6	17	25 0	40	60 0 / 77.5	53	81 1 / 90 6
6—10	62	72 9	91	72 5 / 74 1	98	79 6 / 85 7
10—15	90	81 1	93	87 1 / 87 1	106	83 0 / 87 7
15—20	82	73 2	89	74 2 / 76 4	88	78 4 / 86 4
20—30	52	77 0	94	79 8 / 85 1	118	76 3 / 83 9
30—40	27	70 4	73	89 0 / 87 7	127	90 6 / 90 9
40+	(30+)		114	94 7 / 93 0	123	92 7 / 97 6
Total	638		894		1038	

Note: [illegible] up to 14 years received 3 doses of Salk vaccine, and 62.6% of [illegible] Type 1 vaccine. The [illegible] same children. During [illegible]; 96% from about [illegible] lio strains.

TABLE 8. STATUS OF TYPE 2 ANTIBODY IN THE GENERAL POPULATION OF USTÍ REGION BEFORE AND AFTER THE USE OF SALK VACCINE IN CHILDREN UP TO 14 YEARS OF AGE, AND LIVE VIRUS VACCINE (SABIN) IN CHILDREN 2 TO 8 YEARS OF AGE

	Before Salk Vaccine Early 1957		8 Months After the 3rd Dose of Salk Vaccine, November 1958		2 Months After Administration of Sabin Vaccine Type 2, April 1959	
Age Group Years	Number Tested	Per Cent Positive CPE Test	Number Tested	Per Cent Positive CPE / pH	Number Tested	Per Cent Positive CPE / pH
0—0 5	66	46 0	61	50 8 / 54 1	61	52 5 / 70 5
0 5—1	62	14 5	53	28 3 / 34 0	57	23.1 / 42 1
1—2	65	9 2	51	64 7 / 68 6	61	50 8 / 60 7
2—3	46	34 8	43	62 8 / 69 8	53	66 0 / 79 3
3—4	39	50 2	43	60 5 / 62 8	53	66 0 / 79.3
4—5	31	29 0	49	71 4 / 75 5	40	75 5 / 82 5
5—6	17	37 5	40	85 0 / 87 5	53	83 0 / 94 3
6—10	62	72 9	91	90 0 / 93 0	98	86 7 / 98 0
10—15	90	80 0	93	87 8 / 88 2	106	90.6 / 93 4
15—20	82	73.2	89	78.7 / 83 2	88	77.3 / 88.6
20—30	52	82 7	94	87 2 / 89 4	118	89 1 / 86 4
30—40	27	83 0	73	90 4 / 89 0	127	85 0 / 94 5
40+	(30+)		114	90 4 / 95 6	123	90 2 / 97 6
Total	638		894		1039	

Not ·

etral

TABLE 9. STATUS OF TYPE 3 ANTIBODY IN THE GENERAL POPULATION OF ÚSTÍ REGION BEFORE AND AFTER THE USE OF SALK VACCINE IN CHILDREN UP TO 14 YEARS OF AGE, AND LIVE VIRUS VACCINE (SABIN) IN CHILDREN 2 TO 8 YEARS OF AGE

	Before Salk Vaccine Early 1957		8 Months After the 3rd Dose of Salk Vaccine November 1958		3 Months After Administration of Sabin Vaccine Type 3 Live Virus, April 1959	
Age Group Years	Number Tested	Per Cent Positive CPE Test	Number Tested	Per Cent Positive CPE / pH	Number Tested	Per Cent Positive CPE / pH
0—0 5	66	27 0	61	16 4 / 36 1	61	21 3 / 42 6
0 5—1	62	12 9	53	9 4 / 9 4	57	12 3 / 24 6
1—2	65	27 7	51	25 5 / 33 1	61	16 4 / 31 2
2—3	46	54 3	43	16 8 / 32 6	53	49 9 / 56 6
3—4	38	60 5	43	27 9 / 39 5	53	43 4 / 60 4
4—5	31	71 0	49	44 9 / 63 3	40	62 5 / 65 0
5—6	17	68 7	40	55 0 / 70 0	53	79 3 / 88 7
6—10	62	71 2	91	62 6 / 71 4	98	65 3 / 76 5
10—15	90	79 0	93	77 4 / 91 4	106	71 7 / 84 9
15—20	82	78 0	89	75 3 / 83 2	88	75 0 / 85 2
20—30	52	78 8	94	67 0 / 84 0	118	69 5 / 82 2
30—40	27	77 7	73	78 1 / 88 7	127	67 0 / 86 6
40+	(30+)		114	83 3 / 89 5	123	78 9 / 95 1
Total	638		894		1038	

Note. [illegible] 55 7% of children [illegible] ine. The sera are [illegible] During 1957 only [illegible] In 1958 not one [illegible]

Table 10 Distribution of Naturally Occurring Polioviruses in Czech Regions of Czechoslovakia 1956-1958

(Strains Isolated Mostly from the Patients)

Virus		1956						1957		1958					
						Total		Total						Total	
		I	II	III	IV*	No	%	No	%	I	II	III	IV	No	%
Polio type	1	0	2	8	5	15	50	401	96 3	38	9	82	39	168	91 8
	2	0	0	0	1	1	3 3	0	0	0	1	12	2	15	8 2
	3	0	0	7	7	14	46 6	5	3 7	0	0	0	0	0	
	Total	0	2	15	13	30	100	409	100	57	10	94	41	183	100
ECHO								66		1	2	39	8	50	
Coxsackie								21		0	4	23	11	48	

* Three month period

the increment of antibodies against Type 2 is markedly lower during the above-mentioned month period, i. e., before and after the vaccination with Sabin's vaccine.

It may be of interest that an analogous investigation, the purpose of which is to show the effect of the fourth dose of Salk's vaccine on the state of seroimmunity of the general population in Czechoslovakia, is under way in the Plzen region

Apart from data on the state of seroimmunity in the general population, serological surveys *carried out closely before the vaccination with* live attenuated strains of polioviruses enabled us to estimate the approximate number of children

TABLE 11 PRELIMINARY ESTIMATE OF THE NUMBER OF CHILDREN WITHOUT TYPE 1 ANTIBODY WHO WERE FED TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN)

AGE GROUP YEARS	TYPE 1 ANTIBODY BY PH TEST		NUMBER IN INDICATED AGE GROUP FED TYPE 1 VACCINE	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY
	NUMBER TESTED	% NEGATIVE		
2—3	43	48 8	24,405	11,910
3—4	43	51 2	29,731	15,222
4—5	49	30 6	29,967	9,167
5—6	40	22 5	29,961	6,741
6—8	40	17 5	29,313	5,130
Total	215	34 4	143,377	48,172

TABLE 12. PRELIMINARY ESTIMATE OF THE NUMBER OF CHILDREN WITHOUT TYPE 2 ANTIBODY WHO WERE FED TYPE 2 LIVE POLIOVIRUS VACCINE (SABIN)

AGE GROUP YEARS	TYPE 2 ANTIBODY BY PH TEST		NUMBER IN INDICATED AGE GROUP FED TYPE 2 VACCINE	ESTIMATED NUMBER WITHOUT TYPE 2 ANTIBODY
	NUMBER TESTED	% NEGATIVE		
2—3	43	30 2	19,192	5,196
3—4	43	37 2	23,644	8,196
4—5	49	24 5	23,919	5,860
5—6	40	12 5	23,631	2,954
6—8	40	0	24,124	—
Total	215	21 4	114,510	23,406

TABLE 13 PRELIMINARY ESTIMATE OF THE NUMBER OF CHILDREN WITHOUT TYPE 3 ANTIBODY WHO WERE FED TYPE 3 LIVE POLIOVIRUS VACCINE (SABIN)

AGE GROUP YEARS	TYPE 3 ANTIBODY BY PH TEST		NUMBER IN INDICATED AGE GROUP FED TYPE 3 VACCINE	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY
	NUMBER TESTED	% NEGATIVE		
2—3	43	67 5	21,640	14,611
3—4	43	60 5	26,409	15,977
4—5	49	36 7	26,691	9,795
5—6	40	30 0	26,741	8,022
6—8	40	25 0	25,803	6 451
Total	215	44 2	127,290	54,856

lacking antibodies against the *homologous* virus *before the vaccination*. The results of these calculations are *given* in Table 11 for Type 1, in Table 12 for Type 2, and in Table 13 for Type 3.

From the tables it is apparent that in Czechoslovakia a relatively large percentage of homologously non-immune children were *vaccinated* with attenuated live strains of polioviruses. This applies particularly to Type 1 where cca 35% of the 215 children investigated aged 2-8 years *lacked* antibodies, and to Type 3, where cca 44% of the children *in this* age group were lacking antibodies.

In connection with this finding, we should like *to* point out that for *estimating the* numbers of non-immune children in Tables 11, 12, 13 the *results of serological* investigations of the children in the Ústí *region* were used. In the *serological* surveys made in Czechoslovakia in 1957 *in* three Czech regions (Ústí, *Jihlava*, Praha) in the Ústí *region* the *highest* percentage *incidence* of antibodies against polio *was* revealed for the three regions then investigated, particularly in children. It *is therefore* likely that, on *an average*, in the other regions *where the* live vaccine was administered the percentage of children lacking antibodies *against* the individual types *of* polio viruses was higher, *and* that *more* non-immune children were vaccinated with the attenuated strains than *appears from the* results which we *obtained* in the *Ústí* region. *Information* on these problems will be supplemented after the examination of sera obtained *by* a similar method as *in* Ústí will be available *from* the Jihlava region. This investigation is now in *progress*.

2. *Results of the examination of paired sera samples*

As mentioned in the chapter on materials, we had the *opportunity* to obtain from cca 400 individuals of different ages two *specimens* of serum in November 1958 (one month before the vaccination with Type 1 of the live virus was initiated *in the* Ústí and Jihlava regions) and again in April 1959, i.e., 4, 3, and 2 months after the vaccination with Types 1, 3, and 2 of the attenuated virus. A total of 344 of these pairs of sera were examined quantitatively in five dilutions by *means* of the CPE and pH tests *for neutralizing* antibodies, and by means of the two-dimensional Black and Melnick test and also *for* complement-fixing *antibodies* against all types of polioviruses.

The results of the examination of neutralizing *antibodies*, presented *in detail in* Tables 14, 15 and 16, provide information on the *serological* response in 96 vaccinated children in relation to *their* previous *seroimmunity* and also on the *number of* serologically proved *infections in* 218 persons of different ages and *different previous* states of immunity who had not been vaccinated. It *must* be pointed out that *this group* includes individuals selected at random from the general population of the Ústí and Jihlava regions and *that*, so far, no data are *available* as to whether in *individual* cases of non-vaccinated *subjects* there were family or other contacts.

The most *interesting findings* which emerge *from these results* are that in a *great percentage* of vaccinated children a significant rise of antibodies was recorded, which in homologous non-immune *individuals* amounts to 91.7% *in* Type 1, 83.8% in Type 2, and 85.0% in Type 3. A relatively high percentage of positive serological responses in vaccinated children can be *observed* also in individuals with low antibody levels *prior* to feeding the live viruses (due to vaccination with Salk's vaccine?); in children with a high titer (most probably *of naturally formed* antibodies) the percentage of significant increases *of* antibodies drops considerably.

In non-vaccinated children aged under 10 years, on an average 30-50% positive serological responses were *recorded*, which provide very suggestive evidence of a considerable spread of attenuated polioviruses in the *population*. It is remarkable that *in the* youngest children *aged* 0-2 years, this age group as a whole had a lower percentage of positive serological responses *against* Type 3 than *against* Types 1 and 2, *despite the fact* that in this age group before the vaccination of the population considerably fewer *children* had antibodies against Type 3 than against Types 1 and 2.

3. *Family Studies*

Some *preliminary* results of our yet incomplete virological investigations made in a total of 161 individuals of all age groups in 22 *families* are *summarized* in Tables 17 to 26. A detailed account of the excretion of Types 1 and 3 polio-

TABLE 14. INVESTIGATION OF PAIRED SERUM SPECIMENS OBTAINED FROM VACCINATED AND UNVACCINATED PERSONS IN USTI AND JIHLAVA REGIONS

Development of Type 1 Antibody in Children Who Received Type 1 Live Poliovirus Vaccine (Sabin) and in Unvaccinated Persons of Different Ages According to Antibody Status Prior to Administration of Live Virus Vaccine

Group	Type 1 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 1 Antibody* Number	Per Cent
Vaccinated	Negative	38	36	94 7
(2—8 years)	4—32	22	17	77 3
93 children	64—512	32	17	53 1
(39 6% neg)	>512	4	2	50 0
Unvaccinated	Negative	22	10	45 5
<2 years	4—32	5	2	40 0
34 children	64—512	4	3	75 0
(64 7% neg)	>512	3	1	33 3
	Negative	19	8	42 1
2—6 years	4—32	10	3	33 3
51 children	64—512	18	3	16 6
(37 3% neg)	>512	4	1	25 0
	Negative	6	3	50 0
6—10 years	4—32	7	2	28 6
30 children	64—512	12	0	0
(20 0% neg)	>512	5	0	0
	Negative	27	7	25 9
10 older	4—32	37	5	13 5
143 persons	64—512	46	5	10 9
(18 9% neg)	>512	33	3	9 1

* These data are based on pH and CPE antibody tests on paired serum specimens obtained in Nov 1958, within one month before the first dose of live virus vaccine, and in April 1959, 4 months after feeding of Type 1 vaccine.

viruses in vaccinated and non-vaccinated members of the families in relation to the previous state of immunity (estimated by the pH test) and the rise of homologous antibodies is given in Tables 17 and 18 From these data, which are summarized regardless of the age of the individuals investigated, we can observe a certain direct relationship between the settling and multiplication of attenuated strains of polioviruses of both types and the previous state of seroimmunity: In individuals of different ages fed all three types of attenuated Sabin's polioviruses, all triple negative individuals excrete the virus From persons who before the vaccination lacked homologous antibodies against Types 1 and 3, 75% became infected with both types; the virus was excreted by 45% of individuals with antibodies against Type 1, and 27% of the individuals with antibodies against Type 3, and in individuals who before feeding of the vaccine had antibodies against all three types, the percentage of infected individuals amounts to 20% for Type 1 and 44% for Type 3.

TABLE 15 INVESTIGATION OF PAIRED SERUM SPECIMENS OBTAINED FROM VACCINATED AND UNVACCINATED PERSONS IN ÚSTÍ AND JIHLAVA REGIONS

Development of Type 2 Antibody in Children Who Received Type 2 Live Poliovirus Vaccine (Sabin) and in Unvaccinated Persons of Different Ages According to Antibody Status Prior to Administration of Live Virus Vaccine

Group	Type 2 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 2 Antibody*	
			Number	Per Cent
Vaccinated	Negative	18	15	83.3
(2—8 years)	4—32	41	34	82.9
96 children	64—512	33	12	36.4
(18.7% neg.)	>512	4	0	0
Unvaccinated	Negative	15	6	40.0
<2 years	4—32	13	7	53.8
34 children	64—512	5	2	40.0
(44.1% neg.)	>512	1	1	—
	Negative	14	8	57.1
2—6 years	4—32	18	10	55.5
51 children	64—512	15	6	40.0
(27.4% neg.)	>512	4	0	0
	Negative	1	1	—
6—10 years	4—32	8	2	25.0
30 children	64—512	16	1	6.2
(33.3% neg.)	>512	5	0	0
	Negative	14	5	35.7
10 older	4—32	35	8	22.8
143 persons	64—512	63	11	11.5
(9.8% neg.)	>512	31	1	3.2

* These data are based on pH and CPE antibody tests on paired serum specimens obtained in Nov. 1958 within one month before the first dose of live virus vaccine, and in April 1959, 2 months after feeding of Type 2 vaccine.

Very interesting were the results obtained in investigations of the family contacts (in this case again regardless of age) in relation to the previous state of immunity of the individuals examined: the excretion of Type 1 and 3 viruses was confirmed in 100% of the triple negative individuals; 58 and 55% of those who lacked homologous antibodies became infected with Types 1 and 3; 28 and 20% of those contacts who had homologous antibodies excreted viruses Types 1 and 3. Finally, in immune contacts who prior to the vaccination of family members had antibodies against all three types of polioviruses only 14% of the individuals became infected with Type 3, and 1.7% with Type 1.

These data on the spreading of attenuated polioviruses Type 1 and 3 from vaccinated to non-vaccinated individuals with a different previous state of seroimmunity are summarized in Tables 19 and 20 in relation to the age of the investigated contacts. The preliminary results show that contact infections confirmed by the excretion of the virus in the feces were proved in homologously non-immune children aged

TABLE 14. INVESTIGATION OF PAIRED SERUM SPECIMENS OBTAINED FROM VACCINATED AND UNVACCINATED PERSONS IN ÚSTÍ AND JIHLAVA REGIONS

Development of Type 1 Antibody in Children Who Received Type 1 Live Poliovirus Vaccine (Sabin) and in Unvaccinated Persons of Different Ages According to Antibody Status Prior to Administration of Live Virus Vaccine

Group	Type 1 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 1 Antibody* Number	Per Cent
Vaccinated	Negative	38	36	94 7
(2—8 years)	4—32	22	17	77 3
93 children	64—512	32	17	53 1
(39 6% neg)	>512	4	2	50 0
Unvaccinated	Negative	22	10	45 5
<2 years	4—32	5	2	40 0
34 children	64—512	4	3	75 0
(64 7% neg)	>512	3	1	33 3
	Negative	19	8	42 1
2—6 years	4—32	10	3	33 3
51 children	64—512	18	3	16 6
(37 3% neg)	>512	4	1	25 0
	Negative	6	3	50 0
6—10 years	4—32	7	2	28 6
30 children	64—512	12	0	0
(20 0% neg)	>512	5	0	0
	Negative	27	7	25 9
10 older	4—32	37	5	13 5
143 persons	64—512	46	5	10 9
(18 9% neg)	>512	33	3	9 1

* These data are based on pH and CPE antibody tests on paired serum specimens obtained in Nov. 1958, within one month before the first dose of live virus vaccine, and in April 1959, 4 months after feeding of Type 1 vaccine

viruses in vaccinated and non-vaccinated members of the families in relation to the previous state of immunity (estimated by the pH test) and the rise of homologous antibodies is given in Tables 17 and 18 From these data, which are summarized regardless of the age of the individuals investigated, we can observe a certain direct relationship between the settling and multiplication of attenuated strains of polioviruses of both types and the previous state of sero-immunity: In individuals of different ages fed all three types of attenuated Sabin's polioviruses, all triple negative individuals excrete the virus From persons who before the vaccination lacked homologous antibodies against Types 1 and 3 75% became infected with both types, the virus was excreted by 45% of individuals with antibodies against Type 1, and 27% of the individuals with antibodies against Type 3, and in individuals who *before* feeding of the vaccine had antibodies against all three types, the percentage of infected individuals amounts to 20% for Type 1 and 44% for Type 3.

Table 17 Family Studies

Excretion of Type 1 Attenuated Poliovirus in Vaccinated and Unvaccinated Family Associates According to Antibody Status Prior to Administration of Live Virus Vaccine

Antibodies Prior to Vaccination (pH Test)	Number of Persons Examined	Vaccinated		Number of Persons: Excreting Virus	Ratio	%	Homologous Serologic Response (pH Test)	Ratio	%	Proved Infection: Ratio	%
To all types absent	3	yes	1	1	1/1	100	1	1/1	100	3/3	100
		no	2	2	2/2	100	2	2/2	100		
To Type 1 absent	28	yes	16	12	12/16	75	14	14/16	87	23/28	82
		no	12	7	7/12	58	9	9/12	75		
To Type 1 present	18	yes	11	5	5/11	45	6	6/11	54	8/18	44
		no	7	2	2/7	29	2	2/7	29		
To all three types present	99	yes	41	8	8/41	20	14	14/41	34	18/99	18
		no	58	1	1/58	1 7	4	4/58	7		

Table 18 Family Studies

Excretion of Type 3 Attenuated Poliovirus in Vaccinated and Unvaccinated Family Associates According to Antibody Status Prior to Administration of Live Virus Vaccine

Antibodies Prior to Vaccination (pH Test)	Number of Persons Examined	Vaccinated		Number of Persons: Excreting Virus	Ratio	%	Homologous Serologic Response (pH Test)	Ratio	%	Proved Infection: Ratio	%
To all types absent	3	yes	1	1	1/1	100	1	1/1	100	3/3	100
		no	2	2	2/2	100	2	2/2	100		
To Type 3 absent	25	yes	16	12	12/16	75	11	11/16	69	16/25	64
		no	9	5	5/9	55	5	5/9	55		
To Type 3 present	21	yes	11	3	3/11	27	7	7/11	64	11/21	52
		no	10	2	2/10	20	4	4/10	40		
To all three types present	99	yes	41	18	18/41	44	17	17/41	41	23/99	23
		no	58	8	8/58	14	6	6/58	10		

TABLE 16 INVESTIGATION OF PAIRED SERUM SPECIMENS OBTAINED FROM VACCINATED AND UNVACCINATED PERSONS IN ÚSTÍ AND JIHLAVA REGIONS

Development of Type 3 Antibody in Children Who Received Type 3 Live Poliovirus Vaccine (Sabin) and in Unvaccinated Persons of Different Ages According to Antibody Status Prior to Administration of Live Virus Vaccine

Group	Type 3 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 3 Antibody*	
			Number	Per Cent
Vaccinated	Negative	40	34	85 0
(2—8 years)	4—32	29	19	65 5
96 children	64—512	25	10	40 0
(42 5% neg)	>512	2	0	0
Unvaccinated	Negative	28	5	17 8
<2 years	4—32	5	3	60 0
34 children	64—512	1	0	0
(82 35% neg)	>512	0	0	0
	Negative	20	7	35 0
2—6 years	4—32	22	8	36 4
51 children	64—512	8	1	12 5
(39 2% neg)	>512	1	0	0
	Negative	7	3	42 8
6—10 years	4—32	5	2	40 0
30 children	64—512	17	5	29 4
(23 3% neg)	>512	1	0	0
	Negative	20	4	20 0
10 older	4—32	53	7	13 2
143 persons	64—512	54	6	11 1
(14% neg)	>512	16	0	0

* These data are based on pH and CPE antibody tests on paired serum specimens obtained in Nov 1958 within one month before the first dose of live virus vaccine, and in April 1959, 3 months after feeding of Type 3 vaccine

under 15 in 60% of the cases investigated, while from the vaccinated individuals only 9.4% of persons who previously had antibodies against Type 1 became infected From five successive samples of feces taken at weekly intervals we were not able in a single case to detect virus Type 1 from a total of one non-immune and 31 immune adult family contacts aged above 15 years. In this evaluation, summarized in the above tables, we were able, moreover, to observe in children under 15 years of age a significant antibody response against Type 1, in 86 6% of the non-immune children, and in 18 7% in children with a previous serological immunity ascertained by the pH test

Analogous results for Type 3 are given in Table 20. In non-immune child contacts the virus was isolated in 50% of the cases; and from children which had homologous antibodies in a relatively high number, 27%. From a total of 32 adult contacts investigated, the virus was detected in a single case in an immune person. A significant rise of Type 3 antibodies was recorded in 60% of non immune children, and in

TABLE 21 FAMILY STUDIES

DEVELOPMENT OF TYPE 1 ANTIBODY IN PERSONS OF DIFFERENT AGES WHO RECEIVED TYPE 1 LIVE POLIOVIRUS VACCINE (SABIN) ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

Age Group	Type 1 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 1 Antibody*	
			Number	Per Cent
	Negative	4	4	100.0
0—2 years	4—32	1	1	—
6 children	64—512	1	1	—
(66.6% neg.)	>512	0	0	0
	Negative	8	8	100.0
2—6 years	4—32	4	3	75.0
18 children	64—512	6	5	83.3
(44.4% neg.)	>512	0	0	0
	Negative	2	2	100.0
6—10 years	4—32	4	3	75.0
14 children	64—512	8	4	50.0
(14.2% neg.)	>512	0	0	0
	Negative	4	2	50.0
10 and older	4—32	17	3	17.6
33 persons	64—512	12	2	16.6
(12.1% neg.)	>512	0	0	0

* These data are based on pH and CPE antibody tests on paired serum specimens obtained just before the first dose of live virus vaccine and 3 and a half months after feeding of Type 1 vaccine

TABLE 22. FAMILY STUDIES

DEVELOPMENT OF TYPE 2 ANTIBODY IN PERSONS OF DIFFERENT AGES WHO RECEIVED TYPE 2 LIVE POLIOVIRUS VACCINE (SABIN) ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

Age Group	Type 2 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 2 Antibody*	
			Number	Per Cent
	Negative	0	0	0
0—2 years	4—32	5	5	100.0
6 children	64—512	1	1	—
(0.0% neg.)	>512	0	0	0
	Negative	3	2	66.6
2—6 years	4—32	8	7	87.5
18 children	64—512	5	2	40.0
(16.6% neg.)	>512	2	1	50.0
	Negative	0	0	0
6—10 years	4—32	4	4	100.0
14 children	64—512	7	4	57.1
(0.0% neg.)	>512	3	1	33.3
	Negative	0	0	0
10 and older	4—32	8	7	87.5
33 persons	64—512	25	6	24.0
(0.0% neg.)	>512	0	0	0

* These data are based on pH and SPE antibody tests on paired serum specimens obtained just before the first dose of live virus vaccine and 1 month after feeding of Type 2 [illegible]

TABLE 19. FAMILY STUDIES

TYPE 1—SPREAD OF TYPE 1 VIRUS FROM VACCINATED TO UNVACCINATED ASSOCIATES ACCORDING TO ANTIBODY STATUS

(Preliminary and as yet Incomplete Results)

Family Associates Not Fed Live Virus Vaccine	Total Number	Antibody Status	Total Number	Excr Virus		Significant Rise in Antibody Titer	
				Number Tested	Per Cent	Number Tested	Per Cent
Children	47	Positive	32	3	9 4	6	18 7
		Negative	15	9	60 0	13	86 6
Adults (15+)	32	Positive	31	0	0	3	9 7
		Negative	1	0	0	0	0

TABLE 20 FAMILY STUDIES

TYPE 3—SPREAD OF TYPE 3 VIRUS FROM VACCINATED TO UNVACCINATED ASSOCIATES ACCORDING TO ANTIBODY STATUS

(Preliminary and as yet Incomplete Results)

Family Associates Not Fed Live Virus Vaccine	Total Number	Antibody Status	Total Number	Excr Virus		Significant Rise in Antibody Titer	
				Number Tested	Per Cent	Number Tested	Per Cent
Children	47	Positive	37	10	27.0	14	37 8
		Negative	10	5	50 0	6	60 0
Adults (15 +)		Positive	32	1	3 1	7	22 0
		Negative	0	0	0	0	0

37.8% of the children which had homologous antibodies. In 22% of the adult contacts it also proved possible to confirm the rise of antibodies against Type 3 poliovirus.

In good agreement with these results related to the excretion of polioviruses Types 1 and 3 in vaccinated and non vaccinated family members are the homologous antibody responses; the results are given in detail in Tables 21 to 26

Tables 21, 22, and 23 illustrate the development of neutralizing antibodies against Types 1, [illegible]

vals) according to the antibody status prior to the administration of homologous live virus vaccine Tables 24, 25, and 26 record analogous results obtained in non-vaccinated contacts of different ages in the same families

Thus, from Table 21 it can be clearly seen that all children under 10 years which lacked a previous homologous immunity developed after they were fed the vaccine, antibodies against Type 1 (in all these children the presence of the virus was also confirmed in the feces); Table 23 shows that a 100% antibody response against Type 3 was found only in children aged 0-2 years, while in the age group of children from

TABLE 25. FAMILY STUDIES

DEVELOPMENT OF TYPE 2 ANTIBODY IN FAMILY CONTACTS (UNVACCINATED PERSONS) OF DIFFERENT AGES ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE IN FAMILIES

Age Group	Type 2 Antibody (pH Test) Before Live Virus Vaccination in Families	Number Tested	Significant Rise in Type 2 Antibody*	
			Number	Per Cent
	Negative	0	0	0
0—2 years	4—32	1	1	—
1 child	64—512	0	0	0
	>512	0	0	0
	Negative	3	2	66 6
2—6 years	4—32	2	1	50 0
11 children	64—512	6	1	16 6
(27 2% neg)	>512	0	0	0
	Negative	2	2	100 0
6—10 years	4—32	6	4	66 6
20 children	64—512	12	6	50 0
(10 0% neg)	>512	0	0	0
	Negative	0	0	0
10 and older	4—32	18	4	22 2
46 persons	64—512	27	0	0
(0 0% neg)	>512	1	0	0

* These data are based on pH and CPE antibody tests on paired serum specimens obtained just before the first dose of live virus vaccine was administered to the family associates and one month after feeding of Type 2 vaccine

TABLE 26 FAMILY STUDIES

DEVELOPMENT OF TYPE 3 ANTIBODY IN FAMILY CONTACTS (UNVACCINATED PERSONS) OF DIFFERENT AGES ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE IN FAMILIES

Age Group	Type 3 Antibody (pH Test) Before Live Virus Vaccination in Families	Number Tested	Significant Rise in Type 3 Antibody*	
			Number	Per Cent
	Negative	0	0	0
0—2 years	4—32	1	1	—
1 child	64—512	0	0	0
	>512	0	0	0
	Negative	6	4	66 6
2—6 years	4—32	5	1	20 0
11 children	64—512	0	0	0
(54 5% neg)	>512	0	0	0
	Negative	6	3	50 0
6—10 years	4—32	10	6	60 0
20 children	64—512	4	1	25 0
(30 0% neg)	>512	0	0	0
	Negative	0	0	0
10 and older	4—32	30	5	16 6
46 persons	64—512	15	2	13 3
(0 0% neg)	>512	1	0	0

* These data are based on pH and CPE antibody tests on paired serum specimens obtained just before the first dose of live virus vaccine was administered to the family associates and 2 months after feeding of Type 3 vaccine

TABLE 23. FAMILY STUDIES

DEVELOPMENT OF TYPE 3 ANTIBODY IN PERSONS OF DIFFERENT AGES WHO RECEIVED TYPE 3 LIVE POLIOVIRUS VACCINE (SABIN) ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE

Age Group	Type 3 Antibody (pH Test) Before Live Virus	Number Tested	Significant Rise in Type 3 Antibody*	
			Number	Per Cent
	Negative	3	3	100 0
0—2 years	4—32	3	3	100 0
6 children	64—512	0	0	0
(50 0% neg)	512	0	0	0
	Negative	9	7	77.7
2—6 years	4—32	6	5	83 3
18 children	64—512	3	3	100 0
(50 0% neg)	512	0	0	0
	Negative	1	1	—
6—10 years	4—32	7	5	71 4
14 children	64—512	6	5	83 3
(7 1% neg)	512	0	0	0
	Negative	4	1	25 0
10 and older	4—32	17	6	35 3
33 persons	64—512	12	5	41 6
(12 1% neg)	512	0	0	0

* These data are based on pH and CPE antibody tests on paired serum specimens obtained just before the first dose of live virus vaccine and 2 months after feeding of Type 3 vaccine

TABLE 24 FAMILY STUDIES

DEVELOPMENT OF TYPE 1 ANTIBODY IN FAMILY CONTACTS (UNVACCINATED PERSONS) OF DIFFERENT AGES ACCORDING TO ANTIBODY STATUS PRIOR TO ADMINISTRATION OF LIVE VIRUS VACCINE IN FAMILIES

Age Group	Type 1 Antibody (pH Test) Before Live Virus Vaccination in Families	Number Tested	Significant Rise in Type 1 Antibody*	
			Number	Per Cent
	Negative	0	0	0
0—2 years	4—32	1	0	0
1 child	64—512	0	0	0
	>512	0	0	0
	Negative	4	4	100 0
2—6 years	4—32	3	2	66 0
11 children	64—512	4	1	25 0
(36 3% neg)	>512	0	0	0
	Negative	6	6	100 0
6—10 years	4—32	9	1	11 1
20 children	64—512	5	1	20 0
(30 0% neg)	>512	0	0	0
	Negative	4	1	25 0
10 and older	4—32	26	3	11 5
46 persons	64—512	15	1	6 6
(8 7% neg)	>512	1	0	0

ing of Type

The virological investigation in this child community has not been completed so far, and we are presenting only some preliminary results.

Table 27 gives the results of examinations of the neutralizing antibodies (estimated by the pH test) in samples of serum obtained before vaccination with the live strains (the sera were taken at the beginning of December 1958). It is interesting that the percentage of individuals lacking antibodies against Type 1 poliovirus was at the time relatively high in this community, higher than the average incidence of antibodies in the general population.

During the third week of December 1958 in house A of the institute in Oparany, 17 children aged 2-14 years were vaccinated with 100,000 TCD_{50} Type 1 of the attenuated virus, in house B no one was vaccinated. Apart from the 17 vaccinated children, 37 non-vaccinated children in house A and 24 non-vaccinated children in house B were investigated, as well as several members of the staff from both houses.

The results hitherto obtained of the presence and spread of enteroviruses in this closed community are summarized in Table 28. As can be seen in this table from the (stool) specimens taken before live virus vaccination was initiated, a total of 11 non poliomyelitic cytopathogenic agents were isolated: 10 in house A and one in house B. In the first samples of feces taken from the 17 vaccinated children seven days after they were fed the virus, Type 1 poliovirus was isolated only in one vaccinated child. During subsequent regular weekly investigations in January and February, Type 1 polioviruses were isolated from two to four vaccinated children. In the first half of March, 8 children excreted virus Type 1, in the second half of March three children, in April one vaccinated child excreted the virus, and only in samples of feces from the first half of May was the virus absent in all the 17 vaccinated children.

Non poliomyelitic enteric viruses were isolated in these vaccinated children in two instances in early January, two strains in the first half of March, and one virus in April.

TABLE 28. SPREAD OF TYPE 1 LSc STRAIN OF ATTENUATED POLIO VIRUS AMONG VACCINATED PERSONS AND UNVACCINATED CONTACTS IN THE COMMUNITY OF OPARANY
(Preliminary and as yet Incomplete Results)

Date of the Stool Specimen Sampling (1959)	House A: Number of Persons Excreting Virus Among 17 Vaccinated: Type 1 Polio Virus	Other Enteric Viruses	House A: 37 Unvaccinated: Type 1 Polio Virus	Other Enteric Viruses	House B: Number of Excretors Among 24 Unvaccinated: Type 1 Polio Virus	Other Enteric Viruses
Before vaccination Dec 3d week	0	3	0	7	0	1
After vaccination Dec 4th week	1	0	1	6	0	0
Jan 1st half	4	2	1	5	0	4
2nd half	2	0	3	0	0	0
Feb 1st half	2	0	7	2	0	0
2nd half	4	0	7	1	0	0
March 1st half	8	2	11	0	0	0
2nd half	3	0	10	0	0	0
April 1st half	1	1	10	1	0	1
2nd half	1	0	4	0	0	0
May 1st half	0	0	1	0	0	0
2nd half	—	—	—	—	—	—

2-6 years, two in nine non-vaccinated homologously non-immune children did not develop antibodies against Type 3 In these two children fed Type 3 of the attenuated virus, the virus was not detected in the feces

In individuals above 10 years of age who lacked previously homologous antibodies, we were able to record a significant rise of Type 1 antibodies in 50%, and for Type 3 only in 25% of the individuals It is very interesting that while in individuals above 10 years of age, who prior to the administration of the attenuated viruses had low titers (4-32) of homologous antibodies, a serological response against Type 1 was obtained only in 17.6%, against Type 3 in 35 3%, while a significant rise of antibodies against Type 2 was recorded in 87 5% of the individuals It is likely that these low antibody titers against Type 2 may be due to the vaccination with Salk's vaccine and that there is a higher percentage of these individuals with a humoral vaccination immunity after being fed the live virus than in the case of Type 1, where low antibody levels found before the administration of the second virus probably were due rather to a previous naturally contracted infection than to Salk's vaccine (this applies to individuals above 10 years of age) Thus, a much lower percentage became infected with Type 1 of the attenuated virus *A more detailed explanation of this* finding will be given after the final evaluation of our material (after the investigations of the excretion of Type 2 viruses in the families are completed) and after data on the previous vaccination in different members of the family with Salk's vaccine will have been assembled

The serological investigations of non-vaccinated family contacts according to age are summarized in Tables 24, 25, and 26. From these data *a very high (with one 100% exception)* rate of contact infections with Types 1 and 2 in children under 10 who lacked previous homologous immunity can be noted, while the number of infections with Type 3 in contacts was 66 6% in the age group of 2 6 years, and 50% in the age group 6-10 years. In individuals above 10 years of age (in agreement with the results of the excretion of attenuated viruses) the percentage of secondarily infected family members is again significantly lower in individuals with a previous solid immunity (antibody titers 64-512) in Type 1 it is only 6 6, in Type 2 it drops to zero, and only in Type 3 was there a significant rise of homologous antibodies recorded in 13 3% of the individuals investigated.

4 *Investigations in an Institute for Mentally Defective Children*

As mentioned in the chapter on materials and methods, since December 1958 investigations on the spread of attenuated Type 1 virus are being made also in a closed community of mentally defective children in Oparany. It must be recalled that in this Institute there is a total of cca 400 children and cca 40 adults on the staff living in two large blocks (houses A and B). In our investigation 78 children aged 2-16 years *were included*

Before the investigation was begun, samples of blood and feces were taken from all children investigated and from several members of the staff Subsequently samples of feces taken at weekly intervals were examined for the presence of enteroviruses and blood samples were taken repeatedly from a number of persons, the last in April 1959, from all the children investigated

TABLE 27 THE PRESENCE OF ANTIBODIES AGAINST THREE TYPES OF POLIOVIRUS IN THE COMMUNITY OF OPARANY

Age Groups	Number Tested	Per Cent of Antibodies Against Poliovirus Type 1	2	3	1+2+3	None
2—4	4	0	0	25 0	0	75 0
4—6	14	21 4	78 5	43 0	14 3	14 3
6—10	25	68 0	72 0	64 0	52 0	16 0
10—15	23	56 5	82 6	78 2	56 5	8 6
15+	10	90 0	60 0	70 0	40 0	0

5 *Investigations of the Spread of Enteroviruses in the Population of Different Regions in Czechoslovakia*

Tables 29 and 30 present the results of the isolation of enteroviruses from samples of feces taken at different time intervals before and after the vaccination of children with attenuated live polioviruses had been initiated in the Liberec and Jihlava regions Table 31 summarizes the results obtained in the two regions, where the live poliovaccine was used in Czechoslovakia for vaccinating children aged 2-8 years

As it appears in Table 29, from the 123 samples of feces collected between 27 November and 14 December 1958 in the Liberec region, i e., before the vaccination with the live vaccine had been initiated, no strain of poliovirus was isolated, and so far only one cytopathogenic agent was detected which to date has not been identified From the specimens of feces taken during the second period from 23 January to the end of January 1959, i e., cca 20 days after the vaccination with Type 3 and cca 45 days after the vaccination of the population with Type 1 of the attenuated virus, six strains of Type 3 one strain of Type 2, and in two fecal specimens mixtures of polioviruses were detected During the subsequent period, i e., cca two months after the vaccination with Type 2, three months after the introduction of Type 3, and four months after the introduction of Type 1 into the population of the region, in 243 samples of feces not a single Type 1 poliovirus was found, however, seven Type 3 strains, four Type 2 strains and in one case a mixture of polioviruses were isolated

It is of interest that when the second series of samples was taken, all strains of Types 3 and 2 were detected in vaccinated children (aged under 10 years) and the remaining strain of Type 1 was isolated from a non-vaccinated small child, while in the third series of samples collected after a longer time interval from the total of 12 polioviruses isolated, five were detected in vaccinated and the remaining seven in non-vaccinated children

The results of analogous and simultaneously performed investigations in the Jihlava region (Table 30) are somewhat different, particularly as far as the polioviruses isolated in the third sampling are concerned Although in Jihlava not a single strain of Type 1 was isolated in the third series of samples, despite the fact that in December there were naturally occurring Type 1 polioviruses in the population, contrary to the Liberec region, in Jihlava there was a strikingly low number of Type 2 and 3 strains isolated in the period two to three months after the introduction of these types of live viruses into the population. One single virus Type 3 was detected in a non vaccinated child, and one strain of Type 2 in a vaccinated child As mentioned before, the Jihlava region is a sparsely populated agricultural area, while Liberec, similarly to Ústí, is a densely populated industrial area The results of the third series of the investigations in Jihlava may be, to a certain extent, influenced also by the smaller number of samples (120), as compared with double the number examined in Liberec

It will be useful to summarize the results obtained in the two regions where the vaccination with live poliovaccine was made (Table 31) In the relatively extensive material, it is most striking that in samples obtained cca two weeks after the administration of Type 3 to the population, Type 3 viruses are abundantly found, while during this period, i e , cca one and a half months after the mass vaccination of children with Type 1, practically no polioviruses of Type 1 were found in the population of the two regions, and the spread of these in the population was apparently the result of an interference with Type 3 practically completely suppressed, as suggested also by the results of the investigations of the third sampling taken in April 1959 From the results obtained in the Liberec region it appears, however, that strains of Type 3 persist in the population probably longer, and not even a subsequent vaccination with Type 2 suppressed them within a period of three months. Without going into any premature speculations based on our preliminary results, we wish to draw attention to the fact that the vaccination with Type 3, and particularly Type 2, took place during the already warmer season of the year and, in addition, that the majority of children were vaccinated with Type 1, fewer children with Type 3 and the relatively smallest percentage of children received Type 2 Therefore, in a number of

In the investigated non-vaccinated contacts in house A, no polio virus was present before the investigation was initiated, one week after the feeding of the group of 17 children living in this house, poliovirus Type 1 was isolated from one non-vaccinated child, during the subsequent two weeks also only one strain, in the second half of January three strains; in the first half of February, Type 1 poliovirus was excreted by seven non-vaccinated contacts, in the first half of March the number of excretors was eleven, during the subsequent 30 days virus Type 1 was isolated from 10 children investigated, in the second half of April the number dropped to four and in the first half of May no poliovirus Type 1 was detected in any non vaccinated children living in house A

On the other hand, the number of isolated strains of non-poliomyelitic viruses (the number detected in December 1958 in house A was seven) gradually decreased (6 strains one week after the vaccination was initiated; five strains in the first half of January, two strains in the first, and one strain in the second half of February) and at the end of April and in May in this group of contacts no non-poliomyelitic enteric virus was detected

In house B nobody was vaccinated with the attenuated strain of Type 1 poliovirus and from the 24 children investigated during the period from December 1958 to May 1959 not a single strain of polio virus was isolated. Other non-poliomyelitic viruses were isolated from the feces irregularly. one strain in December, four strains in the first half of January, and one strain in the first half of April.

As far as conclusions can be drawn from the results of the yet incomplete investigation in the institute in Oparany, it is striking that although the state of seroimmunity as regards Type 1 was originally very low in this community, immediately after the vaccination with Type 1 of the attenuated poliovirus in 17 children, only one vaccinated child and one contact excreted the virus In house A, where Type 1 virus was introduced, however, the virus persisted, and during the subsequent relatively long period the number of individuals excreting the virus increased progressively in the vaccinated children but particularly in the direct contacts in this house. The maximum spread of virus Type 1 occurred cca 3 months after the virus had been introduced into the community Parallel with the slow progressive rise of the number of individuals excreting Type 1 poliovirus in house A, the relatively rapid disappearance of non-poliomyelitic enteroviral viruses was observed, though these enteroviruses were fairly numerous in the closed population of mentally defective children It appears thus, that under conditions of a high possibility of fecal contamination, and also under conditions of a considerable incidence of non-poliomyelitic viruses in the intestinal tract, the attenuated Type 1 polio virus was as a result of interference with other enteral viruses able to infect at first only a very small number of vaccinated children and non-vaccinated contacts, including a high percentage of those individuals who prior to vaccination lacked homologous antibodies It is, however, very interesting that the attenuated virus persisted under the given conditions and gradually spread mainly to non vaccinated contacts and gradually suppressed other enteroviruses In this connection, a more precise chronological analysis of the periods when the individual infections developed will be very important, in view of the fact that in the population group repeated passages of the attenuated virus from one person to another and from infected contacts back to the vaccinated children must have occurred Although these latter children did not become infected when fed the virus, after a certain period, however, the virus settled and multiplied

All cca 400 children living in the institute in Oparany were carefully investigated from the very beginning and, so far, not a single case of disease or suspicion of disease of the CNS caused by a virus was recorded The results of repeated serological investigations, and particularly the absence of polioviruses in children in the neighboring house, or any other symptoms, suggest that during the investigation carried out in the quiescent winter period any naturally occurring poliviruses of Type 1 were introduced into the population under observation. At the present time, some of the experiments are being completed and others are under progress. The neurotropic properties of different strains of Type I polioviruses isolated in this investigation from different individuals in different periods of the excretion of the virus will be tested.

5 Investigations of the Spread of Enteroviruses in the Population of Different Regions in Czechoslovakia

Tables 29 and 30 present the results of the isolation of enteroviruses from samples of feces taken at different time intervals before and after the vaccination of children with attenuated live polioviruses had been initiated in the Liberec and Jihlava regions. Table 31 summarizes the results obtained in the two regions, where the live poliovaccine was used in Czechoslovakia for vaccinating children aged 2-8 years.

As it appears in Table 29, from the 123 samples of feces collected between 27 November and 14 December 1958 in the Liberec region, i.e. before the vaccination with the live vaccine had been initiated, no strain of poliovirus was isolated, and so far only one cytopathogenic agent was detected which to date has not been identified. From the specimens of feces taken during the second period from 23 January to the end of January 1959, i.e., cca 20 days after the vaccination with Type 3 and cca 45 days after the vaccination of the population with Type 1 of the attenuated virus, six strains of Type 3, one strain of Type 2, and in two fecal specimens mixtures of polioviruses were detected. During the subsequent period, i.e., cca two months after the vaccination with Type 2, three months after the introduction of Type 3, and four months after the introduction of Type 1 into the population of the region, in 243 samples of feces not a single Type 1 poliovirus was found, however, seven Type 3 strains, four Type 2 strains, and in one case a mixture of polioviruses were isolated.

It is of interest that when the second series of samples was taken, all strains of Types 3 and 2 were detected in vaccinated children (aged under 10 years) and the remaining strain of Type 1 was isolated from a non-vaccinated small child, while in the third series of samples collected after a longer time interval from the total of 12 polioviruses isolated, five were detected in vaccinated and the remaining seven in non-vaccinated children.

The results of analogous and simultaneously performed investigations in the Jihlava region (Table 30) are somewhat different, particularly as far as the polioviruses isolated in the third sampling are concerned. Although in Jihlava not a single strain of Type 1 was isolated in the third series of samples, despite the fact that in December there were naturally occurring Type 1 polioviruses in the population, contrary to the Liberec region, in Jihlava there was a strikingly low number of Type 2 and 3 strains isolated in the period two to three months after the introduction of these types of live viruses into the population. One single virus Type 3 was detected in a non-vaccinated child, and one strain of Type 2 in a vaccinated child. As mentioned before, the Jihlava region is a sparsely populated agricultural area, while Liberec, similarly to Ústí, is a densely populated industrial area. The results of the third series of the investigations in Jihlava may be, to a certain extent, influenced also by the smaller number of samples (120), as compared with double the number examined in Liberec.

It will be useful to summarize the results obtained in the two regions where the vaccination with live poliovaccine was made (Table 31). In the relatively extensive material, it is most striking that in samples obtained cca two weeks after the administration of Type 3 to the population Type 3 viruses are abundantly found, while during this period, i.e., cca one and a half months after the mass vaccination of children with Type 1, practically no polioviruses of Type 1 were found in the population of the two regions, and the spread of these in the population was apparently the result of an interference with Type 3 practically completely suppressed, as suggested also by the results of the investigations of the third sampling taken in April 1959. From the results obtained in the Liberec region it appears, however, that strains of Type 3 persist in the population probably longer, and not even a subsequent vaccination with Type 2 suppressed them within a period of three months. Without going into any premature speculations based on our preliminary results, we wish to draw attention to the fact that the vaccination with Type 3, and particularly Type 2, took place during the already warmer season of the year and, in addition, that the majority of children were vaccinated with Type 1, fewer children with Type 3, and the relatively smallest percentage of children received Type 2. Therefore, in a number of

In the investigated non-vaccinated contacts in house A, no polio virus was present before the investigation was initiated, one week after the feeding of the group of 17 children living in this house, poliovirus Type 1 was isolated from one non-vaccinated child, during the subsequent two weeks also only one strain, in the second half of January three strains, in the first half of February, Type 1 poliovirus was excreted by seven non-vaccinated contacts, in the first half of March the number of excretors was eleven, during the subsequent 30 days virus Type 1 was isolated from 10 children investigated, in the second half of April the number dropped to four, and in the first half of May no poliovirus Type 1 was detected in any non-vaccinated children living in house A

On the other hand, the number of isolated strains of non-poliomyelitic viruses (the number detected in December 1958 in house A was seven) gradually decreased (6 strains one week after the vaccination was initiated, five strains in the first half of January, two strains in the first, and one strain in the second half of February) and at the end of April and in May in this group of contacts no non-poliomyelitic enteric virus was detected

In house B nobody was vaccinated with the attenuated strain of Type 1 poliovirus and from the 24 children investigated during the period from December 1958 to May 1959 not a single strain of polio virus was isolated Other non-poliomyelitic viruses were isolated from the feces irregularly: one strain in December, four strains in the first half of January, and one strain in the first half of April.

As far as conclusions can be drawn from the results of the yet incomplete investigation in the institute in Oparany, it is striking that although the state of seroimmunity as regards Type 1 was originally very low in this community, immediately after the vaccination with Type 1 of the attenuated poliovirus in 17 children, only one vaccinated child and one contact excreted the virus In house A, where Type 1 virus was introduced, however, the virus persisted, and during the subsequent relatively long period the number of individuals excreting the virus increased progressively in the vaccinated children but particularly in the direct contacts in this house The maximum spread of virus Type 1 occurred cca 3 months after the virus had been introduced into the community. Parallel with the slow progressive rise of the number of individuals excreting Type 1 poliovirus in house A, the relatively rapid disappearance of non-poliomyelitic enteroviral viruses was observed, though these enteroviruses were fairly numerous in the closed population of mentally defective children It appears thus, that under conditions of a high possibility of fecal contamination, and also under conditions of a considerable incidence of non poliomyelitic viruses in the intestinal tract, the attenuated Type 1 polio virus was as a result of interference with other enteral viruses able to infect at first only a very small number of vaccinated children and non vaccinated contacts, including a high percentage of those individuals who prior to vaccination lacked homologous antibodies It is, however, very interesting that the attenuated virus persisted under the given conditions and gradually spread mainly to non-vaccinated contacts and gradually suppressed other enteroviruses In this connection, a more precise chronological analysis of the periods when the individual infections developed will be very important, in view of the fact that in the population group repeated passages of the attenuated virus from one person to another and from infected contacts back to the vaccinated children must have occurred Although these latter children did not become infected when fed the virus, after a certain period however, the virus settled and multiplied

All cca 400 children living in the institute in Oparany were carefully investigated from the very beginning and, so far, not a single case of disease or suspicion of disease of the CNS caused by a virus was recorded The results of repeated serological investigations, and particularly the absence of polioviruses in children in the neighboring house, or any other symptoms, suggest that during the investigation carried out in the quiescent winter period any naturally occurring poliviruses of Type 1 were introduced into the population under observation At the present time, some of the experiments are being completed and others are under progress. The neurotropic properties of different strains of Type 1 polioviruses isolated in this investigation from different individuals in different periods of the excretion of the virus will be tested.

5. Investigations of the Spread of Enteroviruses in the Population of Different Regions in Czechoslovakia

Tables 29 and 30 present the results of the isolation of enteroviruses from samples of feces taken at different time intervals before and after the vaccination of children with attenuated live polioviruses had been initiated in the Liberec and Jihlava regions Table 31 summarizes the results obtained in the two regions, where the live polio vaccine was used in Czechoslovakia for vaccinating children aged 2 8 years

As it appears in Table 29, from the 123 samples of feces collected between 27 November and 14 December 1958 in the Liberec region, i e before the vaccination with the live vaccine had been initiated, no strain of poliovirus was isolated, and so far only one cytopathogenic agent was detected which to date has not been identified From the specimens of feces taken during the second period from 23 January to the end of January 1959, i e, cca 20 days after the vaccination with Type 3 and cca 45 days after the vaccination of the population with Type 1 of the attenuated virus, six strains of Type 3, one strain of Type 2, and in two fecal specimens mixtures of polioviruses were detected During the subsequent period i e., cca two months after the vaccination with Type 2, three months after the introduction of Type 3, and four months after the introduction of Type 1 into the population of the region, in 243 samples of feces not a single Type 1 poliovirus was found however seven Type 3 strains, four Type 2 strains, and in one case a mixture of polioviruses were isolated

It is of interest that when the second series of samples was taken, all strains of Types 3 and 2 were detected in vaccinated children (aged under 10 years) and the remaining strain of Type 1 was isolated from a non-vaccinated small child while in the third series of samples collected after a longer time interval from the total of 12 polioviruses isolated, five were detected in vaccinated and the remaining seven in non-vaccinated children

The results of analogous and simultaneously performed investigations in the Jihlava region (Table 30) are somewhat different, particularly as far as the polioviruses isolated in the third sampling are concerned Although in Jihlava not a single strain of Type 1 was isolated in the third series of samples, despite the fact that in December there were naturally occurring Type 1 polioviruses in the population, contrary to the Liberec region, in Jihlava there was a strikingly low number of Type 2 and 3 strains isolated in the period two to three months after the introduction of these types of live viruses into the population One single virus Type 3 was detected in a non vaccinated child, and one strain of Type 2 in a vaccinated child As mentioned before, the Jihlava region is a sparsely populated agricultural area, while Liberec, similarly to Ústí, is a densely populated industrial area The results of the third series of the investigations in Jihlava may be, to a certain extent, influenced also by the smaller number of samples (120) as compared with double the number examined in Liberec

It will be useful to summarize the results obtained in the two regions where the vaccination with live poliovaccine was made (Table 31) In the relatively extensive material, it is most striking that in samples obtained cca two weeks after the administration of Type 3 to the population, Type 3 viruses are abundantly found, while during this period, i e, cca one and a half months after the mass vaccination of children with Type 1 practically no polioviruses of Type 1 were found in the population of the two regions, and the spread of these in the population was apparently the result of an interference with Type 3 practically completely suppressed, as suggested also by the results of the investigations of the third sampling taken in April 1959 From the results obtained in the Liberec region it appears, however, that strains of Type 3 persist in the population probably longer, and not even a subsequent vaccination with Type 2 suppressed them within a period of three months Without going into any premature speculations based on our preliminary results, we wish to draw attention to the fact that the vaccination with Type 3, and particularly Type 2, took place during the already warmer season of the year and, in addition, that the majority of children were vaccinated with Type 1, fewer children with Type 3 and the relatively smallest percentage of children received Type 2 Therefore, in a number of

Table 29. The Spread of Enteroviruses among the Population of Liberec Region in which Live Polio Vaccine (Sabin) Was Used
(Preliminary and as yet Incomplete Results)

		Type 1	Type 3			Type 2		
Date of vaccination		15—21 December 1958	12—17 January 1959			9—14 February 1959		
Date of stool specimens sampling		27 November—14 December 1958	23—29 January 1959			10—22 April 1959		
			Vacc	Unvacc	Total	Vacc	Unvacc	Total
Number of isolated virus strains	Polio Type 1	0	1	0	1	0	0	0
	Polio Type 3	0	6	0	6	2	5	7
	Polio Type 2	0	0	1	1	2	2	4
	Polio Mixture	0	2	0	2	1	0	1
	Other enteric viruses	1	1	0	1	0	2	2

	Type 1				Type 3				Type 2			
Number of stool specimens tested (total)	123				132				243			
Age groups	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group	20	27	39	37	19	20	30	63	66	61	49	67
Number of polioviruses isolated	0				4	5	1	0	5	4	3	0
Number of polioviruses isolated from vacc and unvaccinated persons	0				3/1*	5/0	1/0	0	1/4	3/1	1/2	0

* Numerator=Number of isolated strains of polioviruses from vaccinated persons
Denominator=Number of isolated strains of polioviruses from unvaccinated persons.

Table 30. The Spread of Enteroviruses among the Population of Jihlava Region in which Live Polio Vaccine (Sabin) Was Used
(Preliminary and as yet Incomplete Results)

			Type 1	Type 3			Type 2		
Date of vaccination			15–21 December 1958	12–17 January 1959			9–14 February 1959		
Date of stool specimens sampling			2 December–11 December 1958	23 January–31 January 1959			11–20 April 1959		
				Vacc	Unvacc	Total	Vacc	Unvacc	Total
Number of isolated virus strains	Polio Type	1	2	0	1	1	0	0	0
		3	0	12	6	18	0	1	1
		2	0	0	0	0	1	0	1
	Polio Mixture		0	0	0	0	0	0	0
	Other enteric viruses		5	0	0	0	0	0	0

	Type 1				Type 3				Type 2			
Number of stool specimens tested (total)	119				117				120			
Age groups	0–3	3–6	6–10	10+	0–3	3–6	6–10	10+	0–3	3–6	6–10	10+
Number of stool specimens tested in each group	17	22	19	61	16	17	21	60	20	22	21	57
Number of polioviruses isolated	0	1	1	0	5	8	5	1	1	1	0	0
Number of polioviruses isolated from vacc. and unvaccinated persons	0	0/1	0/1	0	3/2*	8/0	1/4	0/1	0/1	1/0	0	0

* Numerator=Number of isolated strains of polioviruses from the vaccinated persons
Denominator=Number of isolated strains of polioviruses from the unvaccinated persons

TABLE 31. THE SPREAD OF ENTEROVIRUSES AMONG THE POPULATION OF LIBEREC AND JIHLAVA REGIONS IN WHICH LIVE POLIO VACCINE (SABIN) WAS USED

(Preliminary and as yet Incomplete Results)

			Type 1	Type 3			Type 2		
Date of vaccination			15—21 December 1958	12—17 January 1959			9—14 February 1959		
Date of stool specimens sampling			27 November—14 December 1958	23—31 January 1959			10—22 April 1959		
				Vacc	Unvacc	Total	Vacc	Unvacc	Total
Number of isolated virus strains	Polio Type	1	2	1	1	2	0	0	0
		3	0	18	6	21	2	6	8
		2	0	0	1	1	3	2	5
	Polio Mixture		0	2	0	2	1	0	1
	Other enteric viruses		6	1	0	1	0	2	2
Number of stool specimens tested (total)			242	219			363		

	Type 1				Type 3				Type 2			
Age groups	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group	37	19	58	98	35	37	54	123	86	83	70	124
Number of polioviruses isolated	0	1	1	0	9	13	6	1	6	5	3	0
Number of polioviruses isolated from vacc. and unvaccinated persons	0	0/1	0/1	0	6/3*	13/0	2/4	0/1	1/5	4/1	1/2	0

* Numerator=Number of isolated strains of polioviruses from the vaccinated persons.
Denominator=Number of isolated strains of polioviruses from the unvaccinated persons.

TABLE 32 THE SPREAD OF ENTEROVIRUSES AMONG THE POPULATION OF HRADEC REGION IN WHICH LIVE POLIO VACCINE (SABIN) WAS NOT USED (NEIGHBORING REGION OF LIBEREC REGION IN WHICH LIVE VACCINE WAS ADMINISTERED)

(Preliminary and as yet Incomplete Results)

		Type 1				Type 3			
Date of vaccination (in the neighboring region)		15—21 December 1958				12—17 January 1959			
Date of stool specimens sampling		5—15 December 1958				10—11 February 1959			
Number of isolated virus strains	Polio Type 1	0				1			
	Polio Type 3	0				0			
	Polio Type 2	1				0			
	Polio Mixture	0				0			
	Other enteric viruses	3				0			
Number of stool specimens tested (total)		111				93			
Age groups		0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group		33	24	27	27	31	23	16	23
Number of polioviruses isolated		1	0	0	0	0	1	0	0

TABLE 33. THE SPREAD OF ENTEROVIRUSES AMONG THE POPULATION OF C BUDEJOVICE REGION IN WHICH LIVE POLIO VACCINE (SABIN) WAS NOT USED (NEIGHBORING REGION OF JIHLAVA REGION IN WHICH LIVE VACCINE WAS ADMINISTERED)
(Preliminary and as yet Incomplete Results)

		Type 1				Type 3				Type 2			
Date of vaccination (in the neighboring region)		15—21 December 1958				12—17 January 1959				9—14 February 1959			
Date of stool specimens sampling		December 1958—January 1959				February—March 1959				April 1959			
Number of isolated virus strains — Polio Type	1	0				0				2			
	3	0				0				2			
	2	0				0				0			
Polio Mixture		0				0				0			
Other enteric viruses		1				0				5			
Number of stool specimens tested (total)		91				30				161			
Age groups		0—3	3—6	6—10	10+	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group		13	16	28	34	6	13	11	0	44	29	39	49
Number of polioviruses isolated		0	0	0	0	0	0	0	0	1	1	1	1

TABLE 34 THE SPREAD OF ENTEROVIRUSES AMONG THE POPULATION OF GOTTWALDOV REGION IN WHICH LIVE POLIO VACCINE (SABIN) WAS NOT USED (NEIGHBORING REGION OF JIHLAVA REGION IN WHICH LIVE VACCINE WAS ADMINISTERED)
(Preliminary and as yet Incomplete Results)

			Type 1				Type 3			
Date of vaccination in the neighboring region			15—21 December 1958				12—17 January 1959			
Date of stool specimens sampling			3—17 December 1958				10—21 February 1959			
Number of isolated virus strains	Polio Type	1	2				0			
		3	0				0			
		2	0				0			
	Polio Mixture		0				0			
	Other enteric viruses		4				0			
Number of stool specimens tested (total)			91				120			
Age groups			0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group			14	16	23	38	23	16	36	45
Number of polioviruses isolated			0	2	0	0	0	0	0	0

Table 33. The Spread of Enteroviruses among the Population of C. Budejovice Region in which Live Polio Vaccine (Sabin) Was Not Used (neighboring region of Jihlava Region in which live vaccine was administered)
(Preliminary and as yet Incomplete Results)

		Type 1				Type 3				Type 2			
Date of vaccination (in the neighboring region)		15—21 December 1958				12—17 January 1959				9—14 February 1959			
Date of stool specimens sampling		December 1958—January 1959				February—March 1959				April 1959			
Number of isolated virus strains: Polio Type	1	0				0				2			
	3	0				0				2			
	2	0				0				0			
Polio Mixture		0				0				0			
Other enteric viruses		1				0				5			
Number of stool specimens tested (total)		91				30				161			
Age groups		0—3	3—6	6—10	10+	0—3	3—6	6—10	10+	0—3	3—6	6—10	10+
Number of stool specimens tested in each group		13	16	28	34	6	13	11	0	44	29	39	49
Number of polioviruses isolated		0	0	0	0	0	0	0	0	1	1	1	1

Except for a single case where Type 3 virus was isolated from a non-vaccinated individual above the age of 15, it did not prove possible to isolate any viruses of Type 1 and 3, and the percentage of individuals with a positive serological response in this group of adults was also relatively low.

5 Problems of the propagation of Type 1 viruses *were studied in a closed community of mentally* defective children aged 2-16 years. Despite the relatively low percentage of individuals in this community lacking antibodies against Type 1 prior to the vaccination with attenuated viruses (lower than in the average population), of a total of 17 vaccinated children only one child excreted the virus in the feces during the first week after the administration of the virus, during the subsequent 2 months, only 2-4 excreted the virus, three *months after feeding the virus the maximum* number, i.e., 8 children, excreted the virus, four months after the vaccination, one child excreted the virus and only five months after the investigation had been started, the virus was not revealed in a single one of the children investigated.

In the 37 contacts investigated the number of individuals excreting the virus rose so very slowly that the maximum of 11 cases was reached only three months after the *introduction of the virus into the population,* during the 4th month a smaller number of viruses was isolated, and only after five months Type 1 virus *was not isolated from a single child* investigated.

On the other hand, the number of individuals excreting non poliomyelitic viruses in the feces—the number of these viruses prior to the vaccination was seven for the entire community—was gradually reduced during the subsequent period, and four months after the investigation had been started it did not prove possible to detect a single non-poliomyelitic enterovirus.

6 Problems of the incidence of different types of polioviruses in the population (in regions where the vaccination with Sabin's vaccine was carried out as well as those where the vaccine had not been used) were studied and are still being followed up. The results obtained hitherto indicate that from feces col*lected at random from the population of the* two vaccinated regions, within two weeks after vaccination with Type 3, a number of strains of Type 3 poliovirus were isolated, but at present, i.e., cca one and a half months after the introduction of Type 1 into the population, from 249 investigated fecal samples only two strains of Type 1 poliovirus were detected, and after another two months it did prove possible to isolate any Type 1 polioviruses.

Strains of Types 3 and 2 were, however still isolated in a relatively high number in



children who had received virus Type 3 but not virus Type 2, this interference need not have occurred. Investigations of a great number of specimens of feces collected in both regions at the end of May are now under way, and will help to complete the above data, particularly as far as the persistence of Type 3 and 2 strains in the population of the regions where the live vaccine was used is concerned

As mentioned before, the collection of samples of feces and their examination for the presence of enteroviruses were carried out also in three control regions: Hradec Králové, neighboring with the Liberec region, Ceské Budejovice, neighboring with the Jihlava region, and the Gottwaldov region, which is separated from the Jihlava region by another region The results of the investigation of these areas are given in Tables 32, 33, and 34 and show that in the Hradec region not a single Type 1 poliovirus was found in February 1959, that in the Gottwaldov region no poliovirus was isolated in February 1959 (despite the fact that in this region there were naturally occurring polioviruses Type 1 in December), and only in the feces collected in April 1959 in the Budejovice region (neighboring with the Jihlava region), two strains of Type 1 of the virus were isolated, as well as two strains of Type 3 poliovirus So far, however, no information is available regarding their neurotropic and other properties. The problem of the presence of enteroviruses in the population of Czechoslovakia will be elucidated further by the results of examinations of feces made in the first half of June 1959 almost in all regions of Czechoslovakia

Conclusions

1. During the period between December 1958 and February 1959 an experimental vaccination with Sabin's live poliovirus vaccine was carried out in Czechoslovakia, Type 1 was fed to 143,777, Type 3 to 127,290, and Type 2 to 114,510 children aged 2-6 and 8 years, respectively, who were previously vaccinated three times with the inactivated vaccine by the intradermal route. At least 34.5% of these children lacked antibodies against Type 1. Neither during the vaccination with the live vaccine, nor five months after the vaccination was completed, was a rise of polio morbidity or any other adverse phenomenon recorded.
2. In approximately 95% of 96 vaccinated children, which previously lacked antibodies against Type 1, a significant increase of homologous antibodies was revealed. In the remaining two types, the percentage of positive serological responses was somewhat lower (85% against Type 3 and 83% against Type 2) A significant rise of homologous antibodies was recorded in a relatively high percentage of those vaccinated children who had low antibody titers before being fed the live attenuated virus; in children with high titers, the percentage of positive serological responses was markedly reduced, particularly as far as Type 2 and Type 3 are concerned
3. In 248 non-vaccinated individuals of different ages and different states of immunity, as a result of the propagation of attenuated viruses from vaccinated to non-vaccinated individuals, a significant rise of homologous antibody titers was recorded in cca 30-50%, or even a greater proportion in children below the age of 10, particularly in those who previously had no antibodies or only low antibody levels

   In children who previously had high antibody levels and in individuals above the age of 10, the percentage of positive serological responses was considerably lower.
4. The propagation of attenuated viruses from vaccinated to non-vaccinated individuals in relation to age and to the previous state of immunity was investigated in 22 families with many children It was found that in non-vaccinated young contacts (children under 15) Type 1 virus was excreted by homologously non-immune children in 60% and a significant rise of the titers was recorded in 86.6% of the individuals; 94% of the homologously immune contacts excreted virus Type 1 and a serological response was obtained in 18 7% of the individuals

   Similarly, virus Type 3 was isolated in 50% and a rise of the titers was noted in 60% of the non-immune contacts below 15 years of age, in children who had antibodies, the virus was excreted in 27% of the individuals, and in 37% of these contacts it was possible to provide evidence of a significant increase of homologous antibodies.

Except for a single case where Type 3 virus was isolated from a non vaccinated individual above the age of 15, it did not prove possible to isolate any viruses of Type 1 and 3, and the percentage of individuals with a positive serological response in this group of adults was also relatively low.

5 Problems of the propagation of Type 1 viruses were studied in a closed community of mentally defective children aged 2-16 years. Despite the relatively low percentage of individuals in this community lacking antibodies against Type 1 prior to the vaccination with attenuated viruses (lower than in the average population), of a total of 17 vaccinated children only one child excreted the virus in the feces during the first week after the administration of the virus, during the subsequent 2 months, only 2-4 excreted the virus, three months after feeding the virus, the maximum number, i e 8 children, excreted the virus, four months after the vaccination, one child excreted the virus and only five months after the investigation had been started, the virus was not revealed in a single one of the children investigated.

In the 37 contacts investigated the number of individuals excreting the virus rose so very slowly that the maximum of 11 cases was reached only three months after the introduction of the virus into the population, during the 4th month a smaller number of viruses was isolated, and only after five months Type 1 virus was not isolated from a single child investigated.

On the other hand, the number of individuals excreting non-poliomyelitic viruses in the feces—the number of these viruses prior to the vaccination was seven for the entire community—was gradually reduced during the subsequent period, and four months after the investigation had been started it did not prove possible to detect a single non-poliomyelitic enterovirus.

6 Problems of the incidence of different types of polioviruses in the population (in regions where the vaccination with Sabin's vaccine was carried out as well as those where the vaccine had not been used) were studied and are still being followed up. The results obtained hitherto indicate that from feces collected at random from the population of the two vaccinated regions, within two weeks after vaccination with Type 3, a number of strains of Type 3 poliovirus were isolated, but at present, i e, cca one and a half months after the introduction of Type 1 into the population from 249 investigated fecal samples only two strains of Type 1 poliovirus were detected, and after another two months it did prove possible to isolate any Type 1 polioviruses.

Strains of Types 3 and 2 were, however still isolated in a relatively high number in



feces collected two months after the population had been vaccinated with Type 2, and three months after the introduction of Type 3 of the attenuated poliovirus into the population of the two regions

Note The experimental work is still in progress

APPENDIX 1

Record on Control Consultant Examination of Jaroslava Vysínová, born 29 October 1955, address Zitenice No 71, district Litomerice

Family- and case-history With regard to present *disease nothing of interest*

Vaccination

Poliomyelitis
Salk 1—16 5 1957
2—19 6 1957
3— 9 4 1958
Sabin (Type 1)—18 12 1958
Diphtheria, 4 times
BCG +, smallpox +, whooping cough +.

Present illness The child attending a nursery school, had from 28 December 1958, temperatures from 38-39 4°C, a cough, anorexia, headache The temperatures lasted till 1 January 1959, when the temperature dropped At the same time the mother noticed that the child "limped" on its right leg Because this condition did not change, the child was sent to the hospital on 3 January 1959, to the isolation department at Teplice

Condition on admission and course Child limps on right leg, neck two fingers, spine sign +, Lasègue bilat 50°, patellar reflexes bilaterally reduced, more on the right side, reflex of Achilles tendon bilat Ø When walking limps on right leg and walks insecurely also on left leg Recurvation on the right side Spasms Ø

Examination of neurological consultant anteflexion of head against mild resistance, spine sign +, abdominal reflexes reduced (tension) Active motor activity as well as strength of right lower limb slightly reduced RL slightly reduced RLS bilaterally lower (improve after prolonged testing). When walking limps more on right leg, though she is bilaterally paretic.

Summary · Finding very suspicious of poliomyelitis ant. ac. (mild form). Other neurovirosis cannot be ruled out

On 4 and 5 January, condition unchanged 6 January 1959 recurvation is smaller Temperature on admission 37 1°C, later afebrile

Laboratory examination · cerebrospinal fluid El 60/3, S28/3, Ly 32/3, sugars 59 mg %, chlorides 720 mg %.

blood count · Ery 4,650,000, Hb 79, Bi 0 85, L 6,700, Ly 35%, S 50%, T 4%, Eo 9, Mono 2

urinary sediment bacter. ++++, 5-7 L numerous oxalate fragments ++.

liver tests. direct bilirubin Ø, indirect bilirubin 1 0, Takata Ø, Weltman +5, Cd (+), Thymol 1 8, Vidal negative

F W : 18/30.

Examination on 6 January 1959 5 p m..

Girl of normal appearance corresponding to age, adequate nutrition, bad tempered, homesick for mother; willing to cooperate only after some time Sensitive to light, eyelids slightly edemic. During the visual examination searching of special positions of body was not noted

Pupils symmetrical, round in mild mydriasis react well to light The pathways of cerebral nerves are not painful, innervation normal

Neck somewhat stiff, spine sign +, Lasègue on the right cca 45-50°, on the left 65-70°, Amos + When the child sits up (active or passive movement) it selects the resting position of the right hip joint with double flexion of the right lower limb whereby a contraction of the back on the right side and of the flexors of the right lower limb is apparent

Upper limbs finding appears quite normal

Lower limbs patellar reflex on left side very vivid, on the right leg, satisfactory but somewhat lower than on the other side The reflex of the Achilles tendon on the right satisfactory, on the left not produced medioplantar reflex bilat + *Phenomens of pyramid irritation negative, tactile* sensations appear intact. Passive movements in all directions possible, in the hip joint on the right side marked by painful, particularly during adduction and even more during abduction when a striking tension (defensive?) and prominence of the muscle occurs The region of the

hip joint is not painful to touch, during passive dorsal flexion of foot on the right striking elasticity without painful reaction No palpation painfulness During active movements when lying on the back the muscular strength cannot be evaluated, there are however no marked differences between the right and left sides Lateral gluteofemoral ridges on the right and almost vertically to them a keloid scar cca 35 x 2 mm On the planta of the left lower limb a skin affection was treated The skin is everywhere free When child attempted to walk with or without support, after several steps a mild recurvation of the right lower limb was noted, otherwise walking on toes and heels possible

According to the report of Dr Zimák the neurological findings are very variable, even as compared with the morning examination when the patellar reflex was not so vivid and the reflex of the Achilles tendon on the right was readily produced, the child had the right lower limb in a permanently recurved position

Summary Case-history (febrile disease) followed closely by "limping" of child, meningeal syndrome, pathological findings in cerebrospinal fluid suggest the presence of a neuroinfectious disease of virus etiology whereby similar conditions are often found in mild forms of poliomyelitis with mere spasms a temporary adynamia

Dr E Adam
Dr V Adamová

Note The condition of the child returned to normal within the period of compulsory isolation 15 5 1959

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DISCUSSION

Chairman Rhodes: The papers presented by Dr. Voroshilova and Dr Skovránek are open for discussion

Dr. Chumakov *(through an interpreter)* The decision to conduct a large-scale vaccination program with the live poliomyelitis vaccine in our country was taken only after a thorough evaluation of the initial results obtained by Professor Smorodintsev and others

The major significance of our findings of the still-current large-scale vaccination program is based on the principle which they have followed throughout, and that is maximal coverage of the susceptible child population of the area which is under vaccination

In a word, this is the principle of either vaccinating everyone or none

By relying on this principle of massive coverage of the vaccinated area, I feel that several ends can be accomplished one, we will make the population immune at an optimally maximum rapid pace, and two, we will interrupt the chain of the spread of naturally occurring wild polioviruses by removing the basis of its propagation and spread throughout the population

The second peculiarity of the Soviet large-scale vaccination program lies in the fact that we were able to vaccinate over one million children, about one million individuals in a pre-epidemic period, when it was possible to evaluate exactly the effectiveness and safety factors of the vaccine, without the complicating factors of concurrent infection.

In this respect we are contributing nothing new to what has been discussed during this Conference; however, the safety check for us was a most important part of the program, and therefore a great deal of attention and effort was devoted to this aspect of the program.

Because the pre-epidemic period program gave such encouraging results, we are proceeding now with renewed courage, to the immunization of the population on a still larger scale, even though the time of the poliomyelitis season is here.

We realize that, without any question, there will be cases during the poliomyelitis season this year among the vaccinated population This *does not frighten us inasmuch as there are* encouraging results, which we now trust. Because of the careful evaluation of the data, we feel that the vaccine is safe, or safe enough to proceed, in spite of the expected or anticipated occurrence of seasonal poliomyelitis cases

My last remark is that it is too early to talk about the epidemiological effects of the vaccine, because not enough time has been available either to collect information or to analyze it. Most important, the test is going to come in the next few months

The tendency of decreased poliomyelitis morbidity, I believe, is already evident in certain areas of the Soviet Union that have received vaccination.

By this autumn I feel that the data will be available for a detailed presentation and an analysis of the significance of the effectiveness of the current trials

Another little remark. It has not been possible to establish internal controls on the scale on which we were working—to establish internal controls for the administration of the vaccine, by omitting certain portions of the population from vaccine administration

We feel that when the data are completed, it will, nevertheless, be possible to evaluate them by comparison with the incidence of poliomyelitis in the other areas of the Republic, as well as from the carefully collected data on the incidence of poliomyelitis in the now vaccinated area, where the reduction in poliomyelitis morbidity will become evident

Dr Przesmycki In connection with the report of Dr Voroshilova and Dr Smorodintsev, I would like to make a remark. In Wyszkow we vaccinated about 30 per cent of the population We performed our vaccination in October, and from October until today we have had no reported cases of polio.

It seems to me that this experiment shows that the attenuated virus does not change or become pathogenic.

It is only additional proof for the discussion, and especially for the problem mentioned by Dr. Bodian

Of course, it is a small-scale experiment, but it is an experiment that shows that in such conditions the strain of the vaccine did not change.

DR. FOX: I would like to direct this question to either Dr Chumakov or Dr. Smorodintsev

The Russian work has placed a good deal of emphasis on the demonstration of vaccine safety, and I think it has been very impressive in this sense because of the large numbers, relatively speaking, of triple negatives in the populations which were actually fed the vaccine

However, when one thinks about it a moment, the only strain that has been subjected to this test was the first strain fed, namely, the Type 1 strain, because, by definition, if there was any response to the Type 1 strain, the individuals were no longer triple negatives

This may not be a practical question, exactly, but it would be nice to be able to establish the safety of the other strains on the same solid basis as the Type 1, and I am wondering whether the Russians might consider rotating the order in which the strains were fed, in order to give some equivalent information regarding the Type 2 and the Type 3 strains

DR. CHUMAKOV *(through an interpreter)* Such observations have been conducted in the Soviet Union

In the Lithuanian Republic, such an experiment was conducted, dealing with 89,000 vaccinees The vaccine strains were in the following order first, Type 2, then Type 1, and Type 3

There was an additional experiment a much smaller one, instead of 89 000 vaccinees, only 2,700 small children were involved, aged 3 to 5 They received vaccine in still a different order: the first dose was Type 3, then Type 1, and finally, Type 2

In addition of course, there was the large experiment involving 400,000 children, who received a trivalent vaccine

There was no evidence in any of the experiments cited of any untoward effects whatsoever, and therefore I feel that the cumulative effect of all these experiments plus the material reported, adds to the over all impression of safety

DR. SMORODINTSEV. I think the introduction of certain types simultaneously is also a good chance to estimate the safety of this type of vaccine, because experimental and epidemiological investigations have shown that circulation of this virus in the contact groups is very intensive after this introduction, and I suppose that the principal test in the estimation of safety is not only directly vaccinated children, but especially the contact groups, which we reserved in an amount of 20 to 30 per cent of children in the population

The difference in directly vaccinated children is only quantitative. Positive responses to vaccination are a little diminished in such groups But still it is very high, and immunogenic response is better than after triple immunization with killed Salk vaccine

I consider that this method of single immunization with Type 1 virus, and after that the introduction of the divalent vaccine, is pretty good and very convenient for practice

We hope that this information may also be investigated very thoroughly, as perhaps one of the good schedules for immunization with live vaccine

DR. BODIAN I am very glad that Dr Przesmycki brought up the issue of testing for the determination of genetic stability It seems to me his evidence is useful

In addition the immunization program in the Soviet Republic is big enough, so that if we assume there may be persistence of the kind that Dr Skovranek brought up, four months, we could have a wide seeding which would represent the third or even the fourth or later generations of virus progeny seeded in the population

Then the next step is to see what the effect of such seeding might be during an epidemic season, which means the coming summer And in this light, I think that one should look forward with great expectancy to what happens in the Soviet experience, because they have a large-scale trial in a good sized non-immune group, with the epidemic season coming up

So, if we are to find evidence of virus instability in the human population, this would be the time, I should think, provided the epidemic soil is fertile

Now, I want to ask Dr. Chumakov one question in this connection He has just said that he is

certain there will be cases in the coming summer, and if such cases occur in the non-vaccinated population, what are we to do in the way of interpreting the problem of instability?

This is a very difficult problem, and I hope it does not come up I hope we have a less than expected poliomyelitis experience in these populations

But if there is a poliomyelitis experience in the non-vaccinated the vaccinated being immune, we are going to have considerable difficulty in deciding whether we are seeing evidence of protection or evidence of virus instability, or a combination of both.

DR. CHUMAKOV *(through an interpreter)* I wish to state in reply to Dr Bodian's question that during the administration of the vaccine to 1, 200,000 individuals, there were no undesirable effects

I suppose that this is a basis for an evaluation of the cases that may occur during the forthcoming months, which will be made by public health workers, and the scientific workers who can evaluate the individual cases and relate them carefully and in detail as to the administration of the vaccine

It would be extremely difficult to evaluate any particular case, but I believe it can be done.

CHAIRMAN RHODES I understand there is some difficulty in translation

DR SABIN I would like to make a statement for the record, because I think there is a misunderstanding here, and if there is, I would like *to get it straight*

The impression I have is that Professor Chumakov said that he is continuing now in new areas to give the vaccine with full expectancy that the epidemic period will start in those areas and cases will occur, but that he feels justified in doing so, on the basis of the previous experience in non-epidemic times

I had the impression that perhaps Dr Bodian did not catch that during the course of the translation

DR. BODIAN. I did.

CHAIRMAN RHODES. Dr Bodian, would you repeat your question for the record? There is some misunderstanding already, so would you repeat the question, please?

DR. BODIAN: I wonder if Professor Smorodintsev would like to answer this question

CHAIRMAN RHODES: Dr. Smorodintsev, would you like to answer?

DR SMORODINTSEV In many cases in vaccinated or in contact groups during the epidemic period, it is not quite as simple, but I am sure that statistical immunologic studies may indicate the change in the cases with immunization

Live vaccine is not, of course, absolutely effective, there is no reason to believe that these single cases, which may be counted in groups of vaccinated with live vaccine, are necessarily connected with increase of neurovirulence. It is only, of course, that these cases reflect that the vaccine is not 100 per cent effective

The only dangerous thing is the increase of poliomyelitis in vaccinated groups

But if there is a sharp decrease, I think we will have a good chance to calculate these questions more correctly very soon

DR BODIAN I hope there will be a sharp decrease

DR COX My point refers to a question brought up by Dr Henderson, wherein he stated that there was a case of Type 2 poliomyelitis identified in Costa Rica, indicating perhaps that this case was induced by the Type 2 strain used in the vaccination program

Dr Doany informs me he has some pertinent information about this Type 2 case and I would like to have him present it

DR DOANY. In the case reported by Dr Henderson, Type 2 virus was isolated in monkey kidney by Dr Shelokov, and in our laboratory and in both laboratories suckling mice were inoculated with negative results

Knowing the marker of Type 2 virus in Lederle vaccine as being pathogenic for suckling mice, *this might suggest that we are dealing here with* a wild Type 2 virus.

I would like to have Dr Chumakov comment on those results

CHAIRMAN RHODES· Dr Chumakov?

DR CHUMAKOV *(through an interpreter)* The doctor mentioned this to me before we came back to the meeting, and I agreed with him that something should be mentioned, in view of Dr Henderson's earlier remark

CHAIRMAN RHODES: Dr Sabin, would you like to make a more formal statement?

DR SABIN: I have nothing to add

DR MURRAY Perhaps Dr Langmuir should be asking this question, but in view of the fact that such large numbers of persons are involved in the Soviet experience in view of the high percentage of these that were triple negative to start with, these results assume an important element in our consideration of the safety of these products

I think it would be of some interest to us if we knew whether there was any special surveillance mechanism in existence during these studies, or whether the research groups resorted to normal disease reporting systems

CHAIRMAN RHODES Dr Chumakov will answer

DR CHUMAKOV *(through an interpreter)* In the areas of vaccination there is a special and increased surveillance with a number of measures involved All the physicians in the area have been specifically alerted There are teams of investigators who actually investigate each reported case

There is an increased and improved system of reporting and registration, and there is strength in laboratory diagnostic support in these areas

CHAIRMAN RHODES Dr Gear has the floor

DR GEAR While we are discussing safety, this might be an appropriate time to ask a question about the possibility of monkey viruses causing human disease, and to ask those responsible for the manufacture of the vaccine what precaution they take to exclude them

We have heard of some of the tests in which it is possible to exclude baby-mouse pathogenic agents, but there are quite a number of other monkey viruses which are not excluded by these tests

Is it presumed that they are not pathogenic to human beings?

DR CHUMAKOV *(through an interpreter)*: We prepared a lot sufficient for immunization of ten million people, and all the immunizations have been made out of this lot. This was apparently a good vaccine lot, as far as the monkey viruses are concerned

We have followed explicitly Dr Sabin's directions for detection of the possible simian viruses by such measures as leaving uninoculated as much as 10 per cent of the harvest, as control for the appearance of cytopathic effects

During the introduction of live vaccine viruses special attention was paid to this possibility of simian virus contamination As it now stands I feel that for the time being we are fully satisfied with the absence of significant contamination with simian viruses

DR SABIN The idea is to correlate the actual tests that were carried out with the results of the field tests with that material The other test that Dr Gear did not mention that was used was to employ a serum capable of neutralizing about ten million minimal infective doses of the viruses in tissue culture, and to check any virus that broke through that, as to whether it was poliomyelitis or not

If it was not poliomyelitis in testing the undiluted tissue culture fluid you would have another agent This is all that could be done

The other thing that has been suggested for tests that have been carried out is tests in human cells with such neutralizing mixtures as for measles virus, and also tests in primary rabbit tissue culture for which the viruses are not pathogenic, and which will pick up certain adeno-viruses and certain other simian viruses, and also tests in dog kidney, which is not susceptible to poliovirus, but seems to react at least to measles virus

However, in the face of these tests you have the results of the field trials where checks have been made for other minor illnesses

DR GEAR May I ask Dr Sabin? How was the anti-sera against poliovirus prepared? From what?

DR. SABIN. The anti-sera that I used was prepared from monkey kidney tissue culture harvested after 24 hours after inoculation with a massive dose of the virus. Now, the possibility that that might contain a trace of another virus cannot be eliminated, but the probability that it would represent enough antigen to stimulate antibody formation in the rabbits is very unlikely

The way to get such a potent anti-serum was this not to discard the rabbits used in the safety test for B virus, which received large inoculations, and then keep those B virus safety test rabbits for eight months, and if they then get an intravenous booster inoculation, they react with a tremendous booster effect, so that sera of such potency can be prepared

CHAIRMAN RHODES. Dr Hammon.

DR HAMMON In connection with the paper read by Dr Voroshilova, in the very last paragraph I would like to question the meaning of one word with which I think there is some difficulty in translation

The sentence reads "Live virus vaccine from Sabin's strains has been approbated in our country as a safe preparation," and so forth *

I would like to inquire more specifically as to just what is meant by "approbated" Does it mean that the virus has been demonstrated to be safe? Is this the meaning that is wished, that the vaccine has been officially accepted in their country—possibly it has been licensed, and given that type of official approval?

Just what is meant by that?

DR. VOROSHILOVA I think so

INTERPRETER: I will translate, but I do not believe Dr Voroshilova is answering the question, because she did not understand him

CHAIRMAN RHODES: Dr Hammon, would you repeat your question?

INTERPRETER: No. I think I can help Dr Voroshilova in answering it.

The conclusions made here are preliminary, and the final conclusions will be made later

The question really is the word "approbate" as translated from the Russian

CHAIRMAN RHODES: Do you understand the question, Dr Voroshilova?

DR VOROSHILOVA: Perhaps this is a question of language.

INTERPRETER: Yes. Professor Chumakov says it has been tested, with favorable results.

CHAIRMAN RHODES: Tested, with favorable results Dr. Hammon, does that satisfy you?

DR HAMMON. Yes

CHAIRMAN RHODES. Dr Anderson has asked to speak

DR ANDERSON. I would like to get back to Dr Bodian's question, where he raised the point of assuming an outbreak broke out in the Soviet Union How would you know that it was due to a wild virus or to one of the viruses from the vaccine.

As a mere epidemiologist, not a virologist, it would seem to me that one should give some consideration to the question of types involved. If this were an outbreak with all three types, I should suppose we would have to put a great deal of weight upon those types, on the assumption that all three types had escaped, as it were, out into the community

On the other hand, if this were an outbreak of the single type, you would have to suppose either that it was a wild one, or that only one of the three had escaped and the others had had no escape whatsoever.

I realize that that is not as reliable as using some of the bench markers, such as have been discussed the other day, but I think you should give considerable attention to this question of the evidence based on types, because certainly nothing has come up here to indicate in the Sabin vaccine that one of the three types has a much greater capacity to spread out into the community than have the other two.

DR. BODIAN: It seems to me that we are not able to apply the kind of criteria that Dr. Ander-

* See p 529

son has referred to Obviously, if we expected stability to be so poor that all three types could revert to virulent, we would not be here today It seems to me that what we are more likely to face is an improbable escape on the part of one, perhaps, in the course of or following possibly 10, 20, or 50 large field trials

It may be a very small probability that this would happen. It may require that not only the virus escape occur, but that the epidemic soil be fertile.

So that I think that Dr Anderson is going to have to wait a very long time before he sees an epidemic coming up with all three types equally represented

CHAIRMAN RHODES: Dr Sabin?

DR SABIN This comment would be a switch on both Dr Anderson and Dr Bodian I thought Dr Bodian, from his rich experience, would answer it differently

At least my answer would be this that I do not see how you could tell either way, or in a triple way We all know of outbreaks particularly small outbreaks, where Type 1 and Type 2 occur together; we all know of outbreaks where Type 2 and Type 3 might occur together

It is only during the predominantly severe epidemic periods of Type 1 that you have a dominance Therefore I think it would be very difficult to tell, on any of the criteria that you have proposed, Dr Anderson, to get an answer to this question, I don't think this would be the way to approach it.

CHAIRMAN RHODES: Next is Dr Melnick

DR. MELNICK This is a follow-up on the question which Dr Gear raised about simian viruses which might enter into these vaccines

I raise this question which I hope Dr Sabin and Dr Koprowski will answer A simian virus has been reported as contaminant of at least one batch of vaccine that has been used in the Belgian Congo and elsewhere This vaccine, according to Dr. Koprowski's laboratory, was free of simian virus—but when tested in Dr Sabin's laboratory apparently a simian virus was found

I wonder if we could have for the record what tests were done, and how was the agent identified?

CHAIRMAN RHODES: Dr Sabin

DR SABIN This agent was demonstrable in minimal amounts at a dilution of 10^{-2} of the vaccine. It broke down 10 out of 10 tubes with virus that was not poliomyelitis break-through, 7 out of 10 with the 10^{-1} dilution, and 2 out of 10 with the 10^{-2} Tested at the 10^{-1} dilution, there was only Type 1, and no break through

Now here, apparently—the nature of this agent All I can say is that it is an agent that is pathogenic, cytopathogenic for monkey kidney We have not had time to go through the full identification of it. All we can say is that it is not polio 1, 2, or 3

Apparently, material having such a minimal amount of this agent diluted out in the dose that is given, has been administered without as far as I know from what we have heard and the results in Poland and elsewhere, anything that could be attributed to it

While I have the floor, I would like to say one other thing and that is that perhaps to meet an objection that the minimal amounts of an outside agent may be present in monkey kidney tissue culture which we use for immunizing rabbits for future work, I would suggest that poliovirus be grown in HeLa cells, and that the HeLa cell grown virus be used to immunize rabbits to obtain serum That would be an improvement and if I am not mistaken Dr Cabasso has been doing that

CHAIRMAN RHODES Dr Cabasso

DR CABASSO I wanted to comment on Dr Gear's question.

In order to minimize the danger of having any simian virus present in the immunizing antigen we use in rabbits, we think that we have purified our three types of virus by making from 3 to 8 terminal dilution passages in HeLa cells which are not known to support growth of most of the simian viruses We think that in this fashion we are now using, for immunization of rabbits, virus preparations which are essentially free of simian agents

CHAIRMAN RHODES Dr Murray

DR MURRAY This follows up on the question

that was raised by Dr Gear I had hoped to introduce this into the general discussion tomorrow

It seems to us that this question of the ancillary safety factors in connection with the live poliovirus vaccine is one to which we have got to pay great attention The simian agents cannot be brushed off very readily When we look through the manufacturing protocols for killed polio vaccine, we find that great numbers of them occur with great frequency

In fact, there are over 40 of them which are under observation at the present time, not including B virus

In many instances, these things cropped up even when the pool of monkey kidneys consists of two kidneys

B virus is another problem

Well, I may say in passing that this problem has not proved to be of very great difficulty with the killed vaccine, because most of these agents are rather sensitive to formaldehyde, and it is highly improbable that they would be in the vaccine after the formaldehyde has had its effect

But we do not know their relation to human disease, and even in the tests that apply for B virus, we do not know how much B virus is required to infect a human being in relation to the amount that will infect a rabbit

One hopeful thing about B virus is that it is rather labile

CHAIRMAN RHODES Dr Cox

DR COX. Of course we recognize that simian viruses and B virus occasionally may be found, but you must recognize also that the only viruses we can demonstrate at present are those which are cytopathogenic I would like to ask what are you going to do about the viruses which are not cytopathogenic?

All I can say is that thus far we have not encountered a simian or B virus strain to my knowledge I doubt that this is pure luck. I think a great deal of the problem can be controlled by bringing the monkeys in and keeping them in good, isolated quarters, under observation for six to eight weeks, and never introducing a second shipment of monkeys into the same area. I would like to know if Dr. Sabin has ever encountered any B virus or simian viruses in his studies Perhaps we have been lucky so far, but I do not believe it has been entirely a question of luck.

DR. SABIN. In the approximately 75 liters involved in these lots, and also the preparation of another 75 liters previously referred, using Rhesus monkey kidney, these tests that we have used have not revealed any

During my analysis of the data of the large lots prepared by Professor Chumakov's laboratory and Professor Smorodintsev's laboratory, where the same tests have been applied, also none were found

Of course, the number of monkeys necessary to make the large lots here are nothing like the large lots that have been made, of course, of Salk vaccine

DR CABASSO. I would like to add a comment to Dr Murray's question What would we do in a situation like this. The monkey kidney tissue culture is prepared in several containers; one part is seeded with virus and the other left uninoculated for observation for simian agents The virus-infected portion is harvested in three or four days, at which time the portion that has been set aside still looks very good Observation is continued for 14 days and a subculture is made We have, under those circumstances, isolated three simian viruses from that portion which we have left for observation and subculture But we have not been able to isolate the same virus from the harvest containing poliomyelitis virus What can be concluded from this observation? Are not the methods we are now using refined enough to detect the virus in the vaccine preparation? Or is it that simian viruses have no chance to multiply in the three or four days that it takes poliomyelitis virus to develop?

CHAIRMAN RHODES Dr Murray, do you want to reply to that specific comment?

DR MURRAY In the interest of safety we can have only one answer, and that is to throw the batch away

CHAIRMAN RHODES: Dr. Gard?

DR GARD: I would like to make some remarks with regard to Dr Melnick's and Dr. Sabin's

comments on the presence of extraneous viruses in the vaccine used in the Belgian Congo

We have used exactly the same lot for our experiments in Sweden, and this lot was tested for presence of extraneous virus both in Stockholm, and later I have been working with that lot here in the United States, and it has been tested with at least three different Type 1 antisera. They were prepared from monkey kidney cultures, essentially obtained in exactly the same way as Dr Sabin prepares his cultures, that is, 24-hour harvest after a large inoculum, the animal used for immunization is the guinea pig

In the tests we have performed, we have not been able to demonstrate the presence of any foreign virus in that particular lot

I would be very grateful to Dr Sabin if he would let me have one small sample of his particular serum, and also of the strain he relates to that particular lot of virus

DR. SABIN. This requires one little comment and does not take up very much time

I would like to say that the virus was reisolated a second time from the material that we had, using large numbers of control tubes at the same time, and that I still have the bottle I received, and I will be glad to give both the serum and the original bottle to him

CHAIRMAN RHODES Any more comments?

DR. BODIAN: I think one more thing should be said about this whole subject, in view of the exchange that has taken place, and that is that the control of adventitious agents in a live virus vaccine has peculiar difficulties which are yet to be surmounted

CHAIRMAN RHODES: Dr. Gear

DR. GEAR Of course the reason for bringing up this question is that it is one of the most important technical aspects of the preparation of live virus vaccine, for which we do not have the protection given by antiseptics, and we must always recall that accidents with live virus vaccines have resulted from contamination—not necessarily virus vaccines, but accidents with live vaccines have resulted from contamination And it is one of the most important technical aspects of the preparation of such a vaccine

CHAIRMAN RHODES Thank you, Dr Gear

CHAIRMAN RHODES We have arranged the program so that we are just having one more paper today, which is to be given by Dr. Herald Cox on behalf of Dr Oker Blom of Helsinki Finland, "A Small Scale Trial with Live Poliovirus Vaccine in an Isolated Island Community"

15. A SMALL-SCALE TRIAL WITH LIVE POLIOVIRUS VACCINE IN AN ISOLATED ISLAND COMMUNITY

N. OKER-BLOM, HELENA STRANDSTRÖM, AND A. W. ERIKSSON

Department of Virology
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DR COX *(presenting the paper)* Mr. Chairman, ladies and gentlemen

The paper that I am to present is actually the work of Dr Neil Oker-Blom, Miss Helena Strandstrom, and Dr A W Eriksson, in the Department of Virology at the University of Helsinki, Finland

This is a preliminary report, but I think it is rather interesting, and I am sure that later on a more complete report will be forthcoming.

Several field trials with live poliomyelitis vaccines have already been carried out [1 4 6 9-11 13-15] and are at the moment in progress in different parts of the world in accordance with the recommendations of the World Health Organization's Expert Committee on Poliomyelitis [16] The pros and cons of these trials have recently been extensively discussed [5 7 8 12] and among other things the desirability of the continuation of careful small-scale studies has been stressed [6 7]

Such a small-scale trial was initiated on Sottunga Island in the Aland Archipelago, Finland, in January 1958 and is still in progress. The archipelago lies west of the Finnish mainland, and east of Sweden, with the Gulf of Bothnia to the north and the Baltic Sea to the south

For several reasons Sottunga Island seemed to be a suitable region for a small-scale trial with live poliovirus vaccine It is situated in the middle of the archipelago (Fig 1) and, like most islands in this archipelago, is fairly isolated, so that the risk of a spread of the live vaccine virus to other parts of the country seemed small The island has a registered population of only 235 persons, and a still smaller actual population, which facilitated the control of the trial In 1953 the Aland Archipelago was swept by a severe Type 1 poliomyelitis epidemic (paralytic case rate 105 per 100,000), and it was therefore assumed that there would be a fairly good natural immunity, at least to Type 1 poliovirus, throughout the archipelago in inhabitants from the age of 5 years upwards, so that a possible spread of the polio vaccine virus to other parts of the archipelago probably would not be harmful.



FIG 1 Aland Archipelago, Finland

The vaccine selected for use was the SM Type 1 poliovirus developed by Lederle Laboratories, Virus and Rickettsial Research [6 14] It was placed at our disposal by Dr. Herald R Cox, as capsules containing 10^5 tissue culture doses of virus, as estimated by titrations in monkey-kidney tissue.*

This report gives some preliminary data obtained from the first part of the trial.

Plan of the trial The immunity status of the population was determined before the initiation of the trial, and the entire population then received two injections of inactivated poliovirus vaccine at three-week intervals. Six weeks after the second vaccination, blood and stool samples

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were collected from the population, and ten families from different parts of the island (comprising 44 persons) were given live Type 1 poliovirus vaccine (Lederle) orally in capsules. Six and twelve weeks later, blood and stool samples were again collected from the whole population

The intention was to study:

(1) The immunity of the population before vaccination;

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(3) Any occurrence of wild virus in the community, and the spread of the introduced vaccine virus,

(4) The effect of orally administered live poliovirus vaccine on the immunity status of a population group previously partly immunized with inactivated poliovirus vaccine;

(5) Any harmful effect on the community of the introduction of live poliovirus vaccine, and any changes in the neurotropism of the *vaccine virus*

MATERIAL AND METHODS

Virus isolations Stool samples were treated in the conventional way and 0 5 ml inoculated into 3 HeLa cell tubes No titrations of the amount of *virus in stools were made*

Neutralization tests Four-fold dilutions of serum were titrated against about 100 TC doses of virus in HeLa cells The lowest serum dilution used was 1 4, corresponding to a final dilution of 1.8. Part of the sera was titrated at Lederle Laboratories and part at the Department of Virology, University of Helsinki.

Results Final analyses are not yet available, but the results so far obtained may be summarized as follows

1 Although the Aland Archipelago suffered a Type 1 polio epidemic in 1953, Sottunga Island seems to have escaped infection probably owing to its isolated position (Table 1) The findings support the assumption that there has been no polio on Sottunga for the last ten years Of the three positive cases in the age group below ten years, one may have had maternal antibodies and the others may have acquired their infection elsewhere Thus, there apparently is no "wild" virus in the community This assumption is supported by the virus isolation attempts prior to the administration of live poliovirus vaccine

2. Seven weeks after two injections of inactivated poliovirus vaccine, a Type 1 antibody increase was demonstrated in 14 per cent of triple negative persons, in 73 per cent of persons negative to Type 1 only, or to Type 1 and one of the other types, and in 69 per cent of persons already immune to Type 1. An increase in Type 1 antibodies seven weeks after two injections of inactivated poliovirus vaccine was thus demonstrable in altogether about half of the population (Table 2). At this date there remained 39 persons without measurable Type 1 antibodies, of whom 21 were triple negative as estimated by the method used

3 *When live Type 1 poliovirus vaccine* was introduced into this partially immune community where no "wild" poliovirus could be demonstrated, there appeared to be a rapid spread of the virus (Table 3). Six weeks after the administration of the live vaccine, 5 of those who had been given the vaccine still were excreting virus, and virus could also be isolated from another 6 persons Of those who had been vaccinated orally and were excreting virus, three persons were below 10 years of age and two between 10 and 15, and three of them were from the same family In the group not vaccinated orally, three were below 10 years of age and the other three were 11, 22, and 63, respectively Two of these were from the same family Of these 11 persons, three had measurable antibodies against Type 1 prior to the administration of the live poliovirus vaccine, as a result of their vaccination with inactivated poliovirus vaccine The isolated viruses are being tested for neurotropism at Lederle Laboratories, but results are not yet available

4 The administration of live poliovirus vaccine resulted in a Type 1 antibody increase in all the ten persons remaining triple negative and in the single one remaining negative to Type 1 after the vaccination with inactivated *vaccine* Of the persons who did not receive live vaccine and from whom serum samples were collected, 35 out of 102, including four excreting virus, showed an increase in Type 1 *antibodies Such* an increase was observed in 11 out of 21 triple negatives, in 2 out of 8 who had remained negative to Type 1 only or to Type 1 and one of the other types, and in 22 out of the *remaining* 73 having *measurable antibodies* to Type 1 when

15. A SMALL-SCALE TRIAL WITH LIVE POLIOVIRUS VACCINE IN AN ISOLATED ISLAND COMMUNITY

N. OKER-BLOM, HELENA STRANDSTRÖM, AND A. W. ERIKSSON

Department of Virology
University of Helsinki, Finland

DR COX (presenting the paper) Mr. Chairman, ladies and gentlemen·

The paper that I am to present is actually the *work of Dr Neil Oker-Blom*, Miss Helena Strandstrom, and Dr A. W. Eriksson, in the Department of Virology at the University of *Helsinki, Finland*

This is a preliminary report, but I think it is rather interesting, and I am sure that later on a *more complete report will be forthcoming*

Several field trials with live poliomyelitis vaccines have already been carried out [1-4, 6, 8-11, 13, 15] and are at the moment in progress in different parts of the world in accordance with the recommendations of the World Health Organization's Expert Committee on Poliomyelitis.[16] The pros and cons of these trials have recently been extensively discussed [5, 7, 8, 12] and among other things the desirability of the continuation of careful small-scale studies has been stressed.[5, 7]

Such a small-scale trial was initiated on Sottunga Island in the Aland Archipelago, Finland, in January 1958 and is still in progress The archipelago lies west of the Finnish mainland, and east of Sweden, with the Gulf of Bothnia to the north and the Baltic Sea to the south

For several reasons Sottunga Island seemed to be a suitable region for a small-scale trial with live poliovirus vaccine. It is situated in the middle of *the archipelago (Fig. 1) and, like* most islands in this archipelago, is fairly isolated, so that the risk of a spread of the live vaccine *virus to other parts of the country seemed small.* The island has a registered population of only 235 persons, and a still smaller actual population, which facilitated the control of the trial In 1953 the Aland Archipelago was swept by a severe Type 1 poliomyelitis epidemic (paralytic case rate 105 per 100 000), and it was therefore assumed that there would be a fairly good natural immunity, at least to Type 1 poliovirus, throughout the archipelago in inhabitants from the age of 5 years upwards, so that a possible spread of the polio vaccine virus to other parts of the archipelago probably would not be harmful



FIG 1 Aland Archipelago, Finland

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MATERIAL AND METHODS

Virus isolations. Stool samples were treated in the conventional way and 0.5 ml inoculated into 3 HeLa cell tubes. No titrations of the amount of virus in stools were made.

Neutralization tests. Four-fold dilutions of serum were titrated against about 100 TC doses of virus in HeLa cells. The lowest serum dilution used was 1:4, corresponding to a final dilution of 1:8. Part of the sera was titrated at Lederle Laboratories and part at the Department of Virology, University of Helsinki.

Results. Final analyses are not yet available, but the results so far obtained may be summarized as follows:

1. Although the Aland Archipelago suffered a Type 1 polio epidemic in 1953, Sottunga Island seems to have escaped infection, probably owing to its isolated position (Table 1). The findings support the assumption that there has been no polio on Sottunga for the last ten years. Of the three positive cases in the age group below ten years, one may have had maternal antibodies and the others may have acquired their infection elsewhere. Thus, there apparently is no "wild" virus in the community. This assumption is supported by the virus isolation attempts prior to the administration of live poliovirus vaccine.

2. Seven weeks after two injections of inactivated poliovirus vaccine, a Type 1 antibody increase was demonstrated in 14 per cent of triple negative persons, in 73 per cent of persons negative to Type 1 only, or to Type 1 and one of the other types, and in 69 per cent of persons already immune to Type 1. An increase in Type 1 antibodies seven weeks after two injections of inactivated poliovirus vaccine was thus demonstrable in altogether about half of the population (Table 2). At this date there remained 39 persons without measurable Type 1 antibodies, of whom 21 were triple negative as estimated by the method used.

3. When live Type 1 poliovirus vaccine was introduced into this partially immune community where no "wild" poliovirus could be demonstrated, there appeared to be a rapid spread of the virus (Table 3). Six weeks after the administration of the live vaccine, 5 of those who had been given the vaccine still were excreting virus, and virus could also be isolated from another 6 persons. Of those who had been vaccinated orally and were excreting virus, three persons were below 10 years of age and two between 10 and 15, and three of them were from the same family. In the group not vaccinated orally, three were below 10 years of age and the other three were 11, 22, and 63, respectively. Two of these were from the same family. Of these 11 persons, three had measurable antibodies against Type 1 prior to the administration of the live poliovirus vaccine, as a result of their vaccination with inactivated poliovirus vaccine. The isolated viruses are being tested for neurotropism at Lederle Laboratories, but results are not yet available.

4. The administration of live poliovirus vaccine resulted in a Type 1 antibody increase in all the ten persons remaining triple negative and in the single one remaining negative to Type 1 after the vaccination with inactivated vaccine. Of the persons who did not receive live vaccine and from whom serum samples were collected, 35 out of 102, including four excreting virus, showed an increase in Type 1 antibodies. Such an increase was observed in 11 out of 21 triple negatives, in 2 out of 8 who had remained negative to Type 1 only or to Type 1 and one of the other types, and in 22 out of the remaining 73 having measurable antibodies to Type 1 when

Table 1. Incidence of Naturally Occurring Neutralizing Antibodies Against the Three Types of Poliovirus
Sottunga Island, Finland

Age Groups	Total No. of Persons Tested	Number With Neutralizing Antibodies Against						Number of Triple Negative	
		Type 1		Type 2		Type 3			
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
0—1	3	1		—		1		2	
1—2	3	—		—		—		3	
2—3	3	—	6	—	6	—	6	3	90
3—4	5	—		1		—		4	
4—5	5	—		—		—		5	
5—10	12	1		1		1		11	
10—15	27	12	44	6	21	13	48	8	30
15—20	15	12	80	4	27	11	73	1	7
20—30	10	6	60	3	30	5	50	1	10
30—40	31	19	61	22	71	17	55	1	3
40—50	29	26	90	20	72	24	83	—	—
50—60	15	14	93	10	71	15	100	—	—
60 & over	35	24	69	29	83	22	63	2	6
Total	193	115	60%	96	45%	109	56%	41	21%

Table 2 Subjects Showing Increase in Type 1 Poliovirus Antibodies Six Weeks After Date of 2nd Injection of Inactivated Poliovirus Vaccine, Sottunga Island, Finland

Trial Group	Age Group	Antibody Increase, by Pre-Vaccination Status							
		Triple Negative		Negative to Type 1		Positive to Type 1		Total	
		No With Increase	No Pre-Vac	No With Increase	No Pre-Vac	No With Increase	No Pre-Vac	No With Increase	No Pre-Vac
GROUP TO BE FED LIVE VIRUS VACCINE LATER	0—10	2	11	0	0	0	0	2	11
	10—15	0	0	1	1	0	0	1	1
	15 & over	0	1	5	6	13	18	18	25
	TOTAL	2	12	6	7	13	18	21	37
	%	17%		86%		72%		57%	
GROUP NOT TO BE FED LIVE VIRUS VACCINE	0—10	2	12	0	1	1	1	3	14
	10—15	0	8	1	5	3	0	4	19
	15 & over	1	4	15	17	40	60	62	87
	TOTAL	3	24	16	23	50	73	69	120
	%	13%		70%		68%		58%	
TOTAL ALL GROUPS	NO	5	36	22	30	63	91	90	157
	%	14%		73%		69%		57%	

TABLE 3 TYPE 1 POLIOVIRUS ISOLATIONS FROM STOOL SAMPLES OF SUBJECTS BEFORE AND AFTER DATE OF FEEDING OF LIVE TYPE 1 POLIOVIRUS VACCINE (LEDERLE)
SOTTUNGA ISLAND, FINLAND

TOTAL GROUP	ISOLATIONS, BEFORE AND AFTER FEEDING OF TYPE 1 VACCINE					
	BEFORE		6 WEEKS AFTER		12 WEEKS AFTER	
	TOTAL NUMBER	NUMBER POSITIVE	TOTAL NUMBER	NUMBER POSITIVE	TOTAL NUMBER	NUMBER POSITIVE
GROUP FED LIVE VIRUS VACCINE	43	—	37	5*	36	—
GROUP NOT FED LIVE VIRUS VACCINE	158	—	149	6†	148	1‡
TOTAL	201	—	186	11	184	1

* One subject had Type 1 antibodies after vaccination with inactivated vaccine
† Two subjects had Type 1 antibodies after vaccination with inactivated vaccine
‡ This subject also excreted virus 6 weeks earlier

Table 4 Subjects Showing Increase in Type 1 Poliovirus Antibodies Six and/or Twelve Weeks After Date of Feeding of Live Type 1 Poliovirus Vaccine (Lederle)

Trial Group	Age Group	Antibody Increase, by Status Before Feeding Date							
		Triple Negative		Negative to Type 1		Positive to Type 1		Total	
		No With Increase	No Pre-Feeding	No With Increase	No Pre-Feeding	No With Increase	No Pre-Feeding	No With Increase	No Pre-Feeding
GROUP FED LIVE VIRUS VACCINE	0—10	9	9	0	0	2	2	11	11
	10—15	0	0	1	1	2	5	3	6
	15 & over	1	1	0	0	4	21	5	22
	TOTAL	10	10	1	1	8	28	19	39
	%	100%		100%		29%		49%	
GROUP NOT FED LIVE VIRUS VACCINE	0—10	5	10	0	1	1	1	6	12
	10—15	6	8	1	4	3	6	10	18
	15 & over	0	3	1	3	18	66	19	72
	TOTAL	11	21	2	8	22	73	35	102
	%	52%		25%		30%		31%	

live virus vaccine was introduced into the community The antibody studies thus suggest that about one third of the population not receiving live virus vaccine *was in fact infected by the* vaccine virus which had been administered orally to about 20 per cent of the whole population group under study.

5. Because of the geographical position of the island, except in emergency cases, it is visited only once a month by the local doctor from a neighboring island Between these visits, a specially trained nurse is responsible for medical care on the island During the period in question the entire population was medically examined six times by one of the authors During the period 1 April 1958 to 20 May 1959 there were four deaths on the island, among persons aged 58 to 81 One of these died in an accident, two of cardiac insufficiency, and one of cerebral hemorrhage In spite of the fact that there was no autopsy, none of the deaths could be ascribed to poliovirus on the basis of the clinical symptoms *The death rate in 1958-59 corresponded* to the death rate in this population group during the last ten years. No cases of meningitis or paralysis occurred on the island during this period The incidence of acute infections seems to have been of the same order as during previous years Thus no untoward effect ascribable to the introduction of live poliovirus vaccine was observed in the community

Summary Live poliovirus vaccine, Type 1 Lederle, was administered to 44 persons, representing about 20 per cent of an isolated population group partly immunized both naturally and after vaccination with inactivated poliovirus vaccine

In the group receiving the live poliovirus vaccine, a Type 1 antibody response was demonstrated in all of the 11 persons remaining triple negative, or negative to Type 1 only, or to Type 1 and one of the other types after vaccination with the inactivated vaccine

The introduction of the live poliovirus vaccine apparently resulted in a spread of the virus to about 35 per cent of persons not receiving live vaccine, as estimated by virus isolations and antibody titrations.

As far as can be judged clinically, no harmful effect was caused in the persons receiving the live vaccine or in those apparently infected with the virus excreted by these persons.

Regarding the duration of the induced immunity, the persistence of the virus in the community, and any change in neurotropism of this virus, no conclusions can be drawn on the basis of the results so far obtained, but it is hoped that studies in progress will help to elucidate some of these questions

Since the time this paper was prepared, and since it covers only these particular points, the entire population of Sottunga Island has been immunized completely with Types 1, 2, and 3 poliovirus vaccine. The feedings took place somewhat over two months ago, are now completed, and everything is in order.

I have one slide here which may give some insight into what happens to polioviruses, at least to our strains, when they are shed from the human gut on the first and second human passages. This slide happens to be data from experiments carried out, not in Finland, but in the first Minnesota trial of 1957, when they fed 25 newborn infants of 5 days or less and allowed them to go home and live with the family under normal conditions

We tested the feces of both vaccinees and contacts as 10 per cent stool extracts and 20 per cent stool extracts as well as *first* and third tissue culture passages The results of all these tests were made known to our co-workers in Minnesota and the responsible officials of the Pan American Sanitary Bureau before we made any decisions to go ahead with the clinical trials. These data give you some idea at least of what happens to these strains when they are shed through the human gut on the first or second passage.

We examined 10 stools from 7 persons who were fed the vaccine, and 8 stools from their 5 contacts. The tests indicated that 8.3 per cent of monkeys inoculated with a 10 per cent stool extract of vaccinees were paralyzed, as compared with 3 3 per cent for the contacts We used 4 monkeys per group In this column, under "persons fed," the data show 3 monkeys paralyzed out of 36; whereas under "contacts," the data show 1 monkey paralyzed out of 30

Because Dr Dick suggested that a 20 per cent stool suspension would give more desirable data than a 10 per cent suspension, we tried the 20 per

cent suspension on a few stools. Here we see that 11 1 per cent of the monkeys were paralyzed from the vaccinees' stools and 12.2 per cent were paralyzed from the contacts, or 2 out of 18 and 6 out of 49 monkeys were paralyzed

Then, since we had used third passage tissue culture virus from stool isolates in many of our tests, it was suggested that perhaps the virus isolates would be reduced in virulence for monkeys as a result of the tissue culture passages. Accordingly, we tested both third and first tissue culture isolates intracerebrally in monkeys For Type 1, third tissue culture passage, we found the paralysis rate to be 23 8 per cent for the vaccinee stools, and 16 3 per cent for the contact stools, or 24 out of 101 and 15 out of 92 monkeys paralyzed Results with the first tissue culture passage show 24 2 per cent of the monkeys paralyzed by isolates from the vaccinees and 13 8 per cent paralyzed by isolates from the contacts All tissue culture materials were inoculated intracerebrally with at least a million tissue culture doses on a standardized basis These results give no indications, so far as we can see, that the second passage through the human gut increases the activity for monkeys or man If anyone here can see a difference and disagrees, I would like to have it pointed out.

Thank you.

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additional matters about Dr Cox' vaccine tomorrow.

Dr Henderson. Just a very brief question What was the sanitary status of the community? Do you have any idea?

Dr Cox. I cannot answer that question. I presume it must have been on an island where the population was chiefly engaged in fishing but what the sanitary status was, I just do not know

Chairman Rhoads I think we will bring the discussion to a close. The Secretariat asked me to make an important announcement in regard to the program

First of all, we will start at 9 o'clock, and there will be four papers You will note that two have been held over from today, the papers by Dr Embil and Dr Roca Garcia, they will be taken up tomorrow morning, and then the afternoon session will begin at 2 30, when there will be a discussion of a summary of the Conference that is to be prepared

DISCUSSION

CHAIRMAN RHODES The paper presented by Dr. Cox is now open for discussion

DR. BODIAN Since Dr Cox has made such an important issue of the very last table, I would like to say that I do not see how he can interpret that slide at all, unless he knows the titer of virus in the stool material, and I would like also to know how the monkeys were inoculated

It seems to me to be very important in comparing two groups, that you have the tissue culture titers of virus, before comparing the paralytic rate in monkeys.

DR COX: We have on record for every individual the kidney tissue culture titer per gram of stool collected on a weekly basis Furthermore, as I pointed out, these tissue culture materials were inoculated on the basis of 6 logs per culture They were inoculated intracerebrally in the thalamic area with a one-cc dose

DR BODIAN I would like a further clarification, because what I asked was the tissue culture of virus in the stool specimen of the fed and contact groups which produced the 11 and 12 per cent intracerebral paralytic rates, just what were the titers?

DR COX: In other words, we certainly do have records of these data—we collected stools on a weekly basis and each stool was titrated in tissue culture to determine the amount of virus per gram of stool I do not have all the slides with me, but these completed data will be published, so that you can see how much virus was present in the stool per gram at the time it was inoculated into the monkey, either as a 10 per cent or 20 per cent stool extract

All vaccinees were babies and were shedding fairly large quantities of virus. The virus titers were quite high, often nearly 6 logs of virus per gram. Very seldom were low quantities of virus present in the stools except toward the end of the excretion period

CHAIRMAN RHODES: Dr. Bodian, are you satisfied?

DR. BODIAN Yes, we cannot answer the question without the other slides.

CHAIRMAN RHODES: Dr. Dick.

DR DICK. There is just one small point I would like to have clear, and that is in reference to the method which Dr Oker-Blom used to vaccinate his individuals with formalized vaccine Did he follow the technique as used in the Czechoslovakian study, using 0 25 ml and inoculate that intradermally, or did he give his Salk-vaccinated people 1 ml of potent vaccine subcutaneously?

CHAIRMAN RHODES Dr Cox, do you know the answer to that?

DR. COX. I do not I am confident he gave 1 ml doses twice, because we sent the vaccine to him, and the understanding was that we would supply enough inactivated vaccine to give everybody at least 2 cc

CHAIRMAN RHODES Dr Paul

DR PAUL: I would just like to point out that here is another example in Professor Oker-Blom's study how environment conditions the use of this vaccine Apparently, the extra-familial spread involved 35 per cent of susceptible contacts This is not what has happened in a number of other places I am not saying whether it is good or bad It is probably good

CHAIRMAN RHODES Dr. Cox

DR COX I presume it is, Dr. Paul As I said, this is the manuscript that I received

Yesterday in the Minnesota data, of course, it was indicated that the extra familial spread was small

CHAIRMAN RHODES: I think I should point out that there are four papers tomorrow morning, and there will be ample opportunity to raise

additional matters about Dr. Cox' vaccine tomorrow

DR HENDERSON: Just a very brief question What was the sanitary status of the community? Do you have any idea?

DR. COX: I cannot answer that question I presume it must have been on an island where the population was chiefly engaged in fishing, but what the sanitary status was, I just do not know

CHAIRMAN RHODES. I think we will bring the discussion to a close The Secretariat asked me to make an important announcement in regard to the program

First of all, we will start at 9 o'clock, and there will be four papers. You will note that two have been held over from today, the papers by Dr. Embil and Dr. Roca García; they will be taken up tomorrow morning and then the afternoon session will begin at 2 30, when there will be a discussion of a summary of the Conference that is to be prepared

FIFTH SESSION

FRIDAY, 26 JUNE 1959

Chairman

DR. CHARLES ARMSTRONG

Medical Director (ret.)
U. S. Public Health Service
Laboratory of Infectious Diseases
National Institutes of Health
Bethesda, Maryland, U.S.A.

TOPIC V. FIELD TRIALS *(continuation)*

Presentation of Papers by:

Dr. Juan A. Embil

(DISCUSSION)

Dr. Alejandro Guevara Rojas

(DISCUSSION)

Dr. Juan José Leunda

(DISCUSSION)

Dr. Manuel Roca García

(DISCUSSION)

SUMMARY OF THE CONFERENCE



TOPIC V. FIELD TRIALS *(continuation)*

16. A CLINICAL AND SEROLOGICAL STUDY OF THE RESPONSE OF CUBAN CHILDREN TO ORAL VACCINATION WITH ATTENUATED POLIOVIRUS VACCINES

DR. JUAN EMBIL, JR. WITH THE COLLABORATION OF DR. AGUSTÍN CASTELLANOS AND DR. REINALDO MARTÍN JIMÉNEZ *

DR EMBIL *(presenting the paper)* Paralytic poliomyelitis occurs in Cuba as a sporadic early childhood disease with periodic, relatively small outbreaks. Most of the cases are in children under five years of age and less than 15 per cent of the cases are in those over ten years old [1] In the period 1946 to 1957, with the exception of three years the annual number of reported cases varied from 10 to 100 In the years 1946, 1952, and 1955 there were reported respectively 343, 493, and 267 cases [2] Paul, Melnick and Riordan suggest that perhaps only 50 to 75 per cent of the actual cases occurring in Cuba are recorded [3] These authors collected 82 sera in the 1950 survey and demonstrated the presence of Lansing type poliovirus antibodies in 64 per cent of the children from one to four years old and in 75 per cent of those in the five to nine year age group

Our data indicate that all three types of poliovirus are widespread in the Havana metropolitan districts and additional studies are in progress which will greatly expand the surveyed areas and the age groups sampled While Salk-type vaccine was administered to several thousand individuals shortly after this product became available, it has had no national sponsorship and its use has been largely restricted to the practice of a small number of pediatricians and in a few institutions

The use of the Salk type vaccine presents a number of problems for countries whose social and economic conditions are like those of Cuba For this reason and because of the long and successful use of smallpox and yellow fever live virus vaccines in Latin America, the initiation and pursuit of the work with oral attenuated poliovirus vaccines by the Lederle group [4] and the later entrance of Sabin [5] in this field have been followed with great interest

After contact with Dr. H. R Cox of Lederle Laboratories and consultation with Dr F L Soper, then Director of the Pan American Sanitary Bureau, a series of studies was undertaken in Cuba. In our capacity as Director of the Virus Diseases Department of the Municipal Children's Hospital of Havana and as medical advisor for several children's institutions and organizations, it has been possible to explore several aspects of oral poliovirus vaccination in different groups of children under close medical supervision These subjects ranged in age from 1 to 21 years but the majority was between 5 and 12 years old Less than 10 per cent of those vaccinated belong to "open" groups These were dispensary patients on whom follow up work was difficult or impossible in a few instances Most of the children were members of what might be called institutional populations These included several successive groups of underprivileged children who resided in recreation camps for 90-day periods, occupants of a foundling home and an orphanage, as well as children who lived in a Cuban "Boys Town"

and others who attended dormitory schools but often returned home for week ends Observations are being continued on several of these, but serologic data are now complete on the four groups which are the subject of the present report

The vaccines for these studies were provided by the Lederle Laboratories, Division of the American Cyanamid Company, and the serologic assays were done in its Viral and Rickettsial Research Laboratory by methods described elsewhere [6]

OBSERVATIONS

Group I

Since the swallowing of capsules is usually difficult without the aid of fluids, this test was designed to determine whether or not the highly acid carbonated beverages commonly enjoyed by children would inactivate the vaccine virus strains if ingested with the virus containing capsules.

For this trial 16 children, 10 males and 6 females, varying from 4 to 11 years of age were selected They were daily dispensary patients at Mantilla, a suburb of Havana Twelve of the children were white They were residents of multiple-family dwellings in an economically depressed area Many were harboring a variety of intestinal parasites and most of them were undernourished

The capsules containing the vaccine strains of poliovirus were administered at intervals of 21 days in the following order and contained the indicated concentrations of virus per capsule

First Feeding —Type 2—Lot 213B $10^{4.9}$ TCD_{50}
Second Feeding—Type 1—Lot 120G $10^{4.8}$ TCD_{50}
Third Feeding —Type 3—Lot 318B $10^{5.2}$ TCD_{50}

Each child was given approximately 100 cc of a popular Cola-type beverage, having a *pH* of 3.2 with which to wash down the capsules The children were not all started on the program at the same time, but the first feeding was done 19 February and the entire group had received all three strains of virus by 18 April 1958. A blood sample was collected from each child at the time of *the initial feeding* and again 26 to 28 days after administration of the third feeding The sera were *refrigerated until* both the pre- and post-vaccination samples from all children had been obtained. The paired sera from each child were titrated simultaneously. (Tables 1, 2, and 3)

The serological titrations showed that at the time of the first feeding there were 8 of the 16 children who lacked antibody for one or more types of poliovirus The distribution of these *seronegatives was as follows:*

		Total negatives	
Type 1 only	2		
3 only	3		
1, 3	1	Type 1	4
2, 3	1	Type 2	2
1, 2, 3	1	Type 3	6
			12

The post vaccination sera contained but two negatives, both for Type 3 virus, thus the conversion rate was 83%. In addition, 4-fold or greater booster responses were observed in many of the children who possessed antibody *prior to* feeding

The general level of response for each of the three types of poliovirus is indicated by the pre- and post-vaccination geometric mean titers and 4-fold gains which are indicated in Fig 1

GEOMETRIC MEAN ANTIBODY TITERS

Poliovirus Type	*Before*	*After*	*Fold Gain*
1	33	111	3 4x
2	128	412	3 2x
3	15	38	2 5x

The two Type 3 negatives that failed to respond occurred in a 6-year-old who was previously negative only for this strain and a 7-year-old who was negative for both Types 1 and 3 As may be seen in Figure 1 these were the only Type 3 misses in the 6- and 7-year group which included the total of 14 Type 3 negatives. The 7 year-old had excellent responses with both Types 1 and 2, these being from <1:4 to 128 and from 256 to >1024 respectively. Since the other four Type 3 negatives all responded satisfactorily, it cannot be concluded that this virus strain is more readily inactivated than the other strains by an acid beverage such as that employed

The clinical observations on this and the succeeding three groups will be discussed collectively below.

TABLE 1 CUBA—GROUP 1—MANTILLA

POLIOVIRUS TYPE I ANTIBODY RESPONSE OF 16 PERSONS FED ORAL POLIOMYELITIS VACCINE

Distribution of Antibody Titers												Total Number Fed	No. with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4				1			3					4	4
1:4												–	
1:8												—	
1:16													
1:32					1	1				1		3	1
1:64					1	2						3	0
1:128						1	2					3	0
1:256									1			1	0
1:512								1				1	0
1:1024									1			1	
>1:1024												—	
Totals	—	—	—	1	2	4	5	1	2	1	—	16	5

and others who attended dormitory schools but often returned home for week ends Observations are being continued on several of these, but serologic data are now complete on the four groups which are the subject of the present report

The vaccines for these studies were provided by the Lederle Laboratories, Division of the American Cyanamid Company, and the serologic assays were done in its Viral and Rickettsial Research Laboratory by methods described elsewhere[6]

OBSERVATIONS

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The serological titrations showed that at the time of the first feeding there were 8 of the 16 children who lacked antibody for one or more types of poliovirus. The distribution of these seronegatives was as follows:

			Total negatives	
Type 1 only	2			
3 only	3			
1, 3	1		Type 1	4
2, 3	1		Type 2	2
1, 2, 3	1		Type 3	6
				12

The post vaccination sera contained but two negatives, both for Type 3 virus, thus the conversion rate was 83%. In addition, 4-fold or greater booster responses were observed in many of the children who possessed antibody prior to feeding

The general level of response for each of the three types of poliovirus is indicated by the pre- and post-vaccination geometric mean titers and 4-fold gains which are indicated in Fig 1

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The clinical observations on this and the succeeding three groups will be discussed collectively below.

Table 1 *Cuba—Group 1—Mantilla*

Poliovirus Type 1 Antibody Response of 16 Persons Fed Oral Poliomyelitis Vaccine

Pre-Feeding	Distribution of Antibody Titers: Post-Feeding <1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024	Total Number Fed	No. with 4-Fold or Greater Response
<1:4				1			3					4	4
1:4													
1:8													
1:16													
1:32					1	1				1		3	1
1:64					1	2						3	0
1:128						1	2					3	0
1:256									1			1	0
1:512								1				1	0
1:1024									1			1	
>1:1024													
Totals	—	—		1	2	4	5	1	2	1	—	16	5

TABLE 2. CUBA—GROUP 1—MANTILLA
POLIOVIRUS TYPE 2 ANTIBODY RESPONSE OF 16 PERSONS FED ORAL POLIOMYELITIS VACCINE

Distribution of Antibody Titers													
Pre-Feeding	Post-Feeding											Total Number Fed	No. with 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			2									2	2
1:4										1		1	1
1:8			1									1	0
1:16												—	
1:32									1			1	1
1:64												—	
1:128						1			2		1	4	3
1:256									2		1	3	1
1:512							1		1			2	0
1:1024										2		2	
>1:1024												—	
Totals	—	—	3	—	—	1	1	—	6	3	2	16	8

Table 3 Cuba—Group 1—Mantilla

Poliovirus Type 3 Antibody Response of 16 Persons Fed Oral Poliomyelitis Vaccine

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding											Total Number Fed	No with 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2	1	2				1					6	4
1:4												—	
1:8											1	1	1
1:16												—	
1:32	1				1		1		1			4	2
1:64						1						1	0
1:128												—	
1:256							2		1			3	0
1:512								1				1	0
1:1024												—	
>1:1024												—	
Totals	3	1	2	—	1	1	4	1	2	—	1	16	7

Feedings with Coca Cola, pH 3 2

No of Subjects· 16

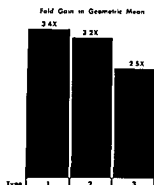
Ages of Subjects 4 yrs —3 8 yrs —2 10 yrs —2
6 yrs —3 9 yrs —1 11 yrs —1
7 yrs —4

Antibody Titers
Geometric Mean by Type

	1	2	3
Pre-vaccination	33	128	15
Post-vaccination	111	412	38

Antibody Negatives

Total number	12
No. responding	10
Percent responding	83%



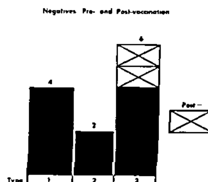


FIG 1 Oral Poliomyelitis Vaccine Cuba—Group 1—Mantilla

Group II

At the suggestion of Dr H R. Cox it was decided to reinvestigate the problem of feeding all three virus strains simultaneously. For this purpose, 62 children were selected. Ages ranged from 4 to 17 years, the median being 10 All of the children were female Fifty five were white They were residents of what may be described as a dormitory school in the environs of Havana The girls lived in the institution but were free to return to their homes for weekend holidays every three to four weeks The school was operated by the church and many of the children held scholarships contributed by various charitable organizations The students represented families of modest incomes and they were healthy and well nourished

All of the children were fed on 13 May 1958, at which time a blood sample was collected from each. Post vaccination sera were obtained 38 days later. The same three lots of vaccine employed in the preceding group were used in this trial. Each child received one capsule of Type 1 poliovirus, one of Type 3, and two Type 2 virus simultaneously, 30 minutes after a meal. The swallowing of the capsules was facilitated by the ingestion of approximately 100 cc. of a suspension of non-fat dry milk solids (Star-lac). The girls were under the daily observation of nuns and were visited at frequent intervals by the staff physician

Serum neutralization tests on the pre-vaccination sera revealed 36 sero-negatives among the 62 children They were distributed in the following combinations:

Poliovirus Type		Total negatives	
1	1		
3	12		
1, 2	2	Type 1	11
1, 3	7	Type 2	4
2, 3	1	Type 3	21
1, 2, 3	1		36

The post-vaccination serum studies disclosed four residual negatives. An 11-year old, negative only to Type 1 prior to vaccination, and a 12-year old who had been negative for both Types 1 and 2 had both failed to respond to Type 1 strain The other two misses were for Type 3 virus, one in an 11-year old negative only for Type 3 and the other in a 14-year old, who had been without antibodies for Types 2 and 3 before the simultaneous feeding of all three virus strains. The conversion rate for the pre-feeding negatives was 89 per cent and in addition 21 of 50 children whose antibody titers had ranged from 1 to 4 and 1 to 512 in their pre-vaccina

Table 4 Cuba—Group 2—College

Poliovirus Type 1 Antibody Response of 62 Persons to Simultaneous Feedings of Oral Poliomyelitis Vaccine

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding											Total Number Fed	No with 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2		1	2	4	1	1					11	9
1:4								1				1	1
1:8				1	1		1	1	1			5	4
1:16					1	1			1			3	2
1:32					4	1	3		1			9	4
1:64					1	1	1	1	1	1		6	3
1:128						2	5	2	3	1		13	4
1:256							3		1		2	6	2
1:512								1	4	1	1	7	1
1:1024										1		1	
>1:1024												—	
Totals	2		1	3	11	6	14	6	12	4	3	62	30

TABLE 5 CUBA—GROUP 2—COLLEGE

POLIOVIRUS TYPE 2 ANTIBODY RESPONSE OF 62 PERSONS TO SIMULTANEOUS FEEDINGS OF ORAL POLIOMYELITIS VACCINE

	DISTRIBUTION OF ANTIBODY TITERS												
PRE-FEEDING	POST-FEEDING											TOTAL NUMBER FED	NO WITH 4-FOLD OR GREATER RESPONSE
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			2	1			1					4	4
1:4						1						1	1
1:8			1	1								2	0
1:16					1							1	0
1:32					1	2		1	1		2	7	4
1:64									1		1	2	2
1:128							2	1	4		2	9	6
1:256								1	2	3	3	9	6
1:512								1	8	4	5	18	5
1:1024									1	1		2	
>1:1024									1		6	7	
Totals	—	—	3	2	2	3	3	4	18	8	19	62	28

Table 6 Cuba—Group 2—College

Poliovirus Type 3 Antibody Response of 62 Persons to Simultaneous Feedings of Oral Poliomyelitis Vaccine

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding											Total Number Fed	No with 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2	1	5	2	1	1	3	1	3	1	1	21	19
1:4			2				1					3	1
1:8			1		1		1		2			5	4
1:16				1	1						1	3	1
1:32					4	1		2	3	1	1	12	7
1:64							2		1	1		4	2
1:128						1	2	1	1	1	1	7	3
1:256							2					2	0
1:512								1		1	1	3	1
1:1024												—	
>1:1024											2	2	
Totals	2	1	8	3	7	3	11	5	10	5	7	62	38

TABLE 5 CUBA—GROUP 2—COLLEGE

POLIOVIRUS TYPE 2 ANTIBODY RESPONSE OF 62 PERSONS TO SIMULTANEOUS FEEDINGS OF ORAL POLIOMYELITIS VACCINE

Distribution of Antibody Titers												Total Number Fed	No with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			2	1			1					4	1
1:4						1						1	1
1:8			1	1								2	0
1:16					1							1	0
1:32					1	2		1	1		2	7	4
1:64									1		1	2	2
1:128							2	1	4		2	9	6
1:256								1	2	3	3	9	6
1:512								1	8	4	5	18	5
1:1024									1	1		2	
>1:1024									1		6	7	
Totals		-	3	2	2	3	3	4	18	8	19	62	28

Table 7. Cuba—Group 3—Matanzas City

Poliovirus Type 1 Antibody Response of 91 Persons Fed Oral Poliomyelitis Vaccine in 3 Feedings, 1 Week Apart

Distribution of Antibody Titers												Total Number Fed	No. with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	1		2		4	2	1					10	9
1:4												—	—
1:8					2							2	2
1:16				1	2	1		1				5	2
1:32				2	9	4	2	2				19	4
1:64					2	3	3					8	0
1:128						1	5	2	8	1	1	18	10
1:256								4	3	1	1	9	2
1:512								2	6	1	1	10	1
1:1024									1		3	4	
>1:1024									1		5	6	
Totals	1	—	2	3	19	11	11	11	19	3	11	91	30

Feedings: Simultaneous

No. of Subjects 62

Ages of Subjects 4 yrs—1 7 yrs—7 10 yrs—9 13 yrs—4
5 yrs—4 8 yrs—2 11 yrs—7 14 yrs—6
6 yrs—5 9 yrs—7 12 yrs—7 15 yrs—1
17 yrs—2

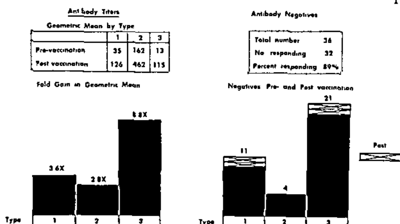


FIG. 2 Oral Poliomyelitis Vaccine Cuba—Group 2—College

tion sera manifested 4-fold or greater gains in titer The detailed responses to each of the three virus strains are summarized in Tables 4, 5, and 6 The composite antibody gains are reflected in the geometric mean titers before and after simultaneous virus feedings

GEOMETRIC MEAN ANTIBODY TITERS

Poliovirus Type	*Before*	*After*	*Fold Gain*
1	35	126	3 6x
2	162	462	2 8x
3	13	115	8 8x

It is of interest to note that the triple negative in this group a 10-year old girl, responded with titers of 128, 16, and 1024 for Types 1, 2, and 3, respectively

Group III

In order to gain some information regarding the flexibility of the feeding schedule, 91 children were fed the three strains of poliovirus with a 7-day interval between the ingestion of each type The medium age of the children was 8 years and the range was 6 to 11 Seventy two of the children were white Fifty were male. All were residents of the Matanzas City Recreation Camp. They were heavily parasitized and came from the poorest economic strata of the rural areas where malnutrition is a way of life. The children stayed for 90 days in the recreation camp and during this time they were well fed and cared for There is a resident physician at the camp and his primary activity is to restore the health of the children, rid them of parasites and return them to their homes in good physical condition The camp is approximately 75 miles east of Havana The first feeding was two capsules of Type 2 virus containing $10^{5.8}$ TCD_{50} of virus on 6 September 1958 A week later the children were fed two capsules of Type 3 virus containing $10^{5.6}$ TCD_{50}, and the final feeding was $10^{5.5}$ TCD_{50} of Type 1 virus in a single capsule At each feeding the children were given a glass of fat-free dry milk with a pH of 6 7 Serum samples were collected at the time of the first feeding and 34 days after the administration of the Type 1 strain

At the time of the primary feeding there were 23 seronegatives distributed among 16 children as follows:

Poliovirus Type	*Number*	*Total Negatives*	
1	5	Type 1	10
2	1	Type 2	3
3	3	Type 3	10
1, 3	5		—
2, 3	2		23

Post vaccination serum neutralization tests showed that the negative to positive conversion rate was 96 per cent A single Type 1 negative remained This was in a 9-year-old girl who had previously been without antibodies for both Types 1 and 3 poliovirus; her Type 2 titer re-

Table 7 Cuba—Group 3—Matanzas City

Poliovirus Type 1 Antibody Response of 91 Persons Fed Oral Poliomyelitis Vaccine in 3 Feedings, 1 Week Apart

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding: <1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024	Total Number Fed	No. with 4-Fold or Greater Response
<1:4	1		2		4	2	1					10	9
1:4												—	—
1:8					2							2	2
1:16				1	2	1		1				5	2
1:32				2	9	4	2	2				19	4
1:64					2	3	1					8	0
1:128						1	5	2	8	1	1	18	10
1:256								4	3	1	1	9	2
1:512								2	6	1	1	10	1
1:1024									1		3	4	
>1:1024									1		5	6	
Totals	1	—	2	3	19	11	11	11	19	3	11	91	30

Feedings Simultaneous

No of Subjects 62

Ages of Subjects 4 yrs—1 7 yrs—7 10 yrs—9 13 yrs—4
5 yrs—4 8 yrs—2 11 yrs—7 14 yrs—6
6 yrs—5 9 yrs—7 12 yrs—7 15 yrs—1
17 yrs—2

Antibody Titers

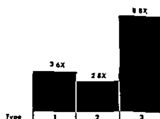
Geometric Mean by Type

	1	2	3
Pre-vaccination	35	162	13
Post vaccination	126	462	115

Antibody Negatives

Total number	36
No responding	32
Percent responding	89%

Fold Gain in Geometric Mean



Negatives Pre- and Post vaccination

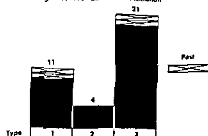


FIG 2 Oral Poliomyelitis Vaccine Cuba—Group 2—College

tion sera manifested 4-fold or greater gains in titer The detailed responses to each of the three virus strains are summarized in Tables 4 5, and 6 The composite antibody gains are reflected in the geometric mean titers before and after simultaneous virus feedings

GEOMETRIC MEAN ANTIBODY TITERS

Poliovirus Type	*Before*	*After*	*Fold Gain*
1	35	126	3 6x
2	162	462	2 8x
3	13	115	8 8x

It is of interest to note that the triple negative in this group, a 10-year old girl, responded with titers of 128, 16, and 1024 for Types 1, 2, and 3, respectively

Group III

In order to gain some information regarding the flexibility of the feeding schedule, 91 children were fed the three strains of poliovirus with a 7-day interval between the ingestion of each type The medium age of the children was 8 years and the range was 6 to 11. Seventy-two of the children were white. Fifty were male All were residents of the Matanzas City Recreation Camp They were heavily parasitized and came from the poorest economic strata of the rural areas where malnutrition is a way of life. The children stayed for 90 days in the recreation camp and during this time they were well fed and cared for There is a resident physician at the camp and his primary activity is to restore the health of the children, rid them of parasites and return them to their homes in good physical condition. The camp is approximately 75 miles east of Havana The first feeding was two capsules of Type 2 virus containing $10^{5.3}$ TCD_{50} of virus on 6 September 1958 A week later the children were fed two capsules of Type 3 virus containing $10^{5.6}$ TCD_{50}, and the final feeding was $10^{5.5}$ TCD_{50} of Type 1 virus in a single capsule At each feeding the children were given a glass of fat-free dry milk with a pH of 6 7 Serum samples were collected at the time of the first feeding and 34 days after the administration of the Type 1 strain

At the time of the primary feeding there were 23 seronegatives distributed among 16 children as follows·

Poliovirus Type	*Number*	*Total Negatives*	
1	5	Type 1	10
2	1	Type 2	3
3	3	Type 3	10
1, 3	5		—
2, 3	2		23

Post vaccination serum neutralization tests showed that the negative to positive conversion rate was 96 per cent A single Type 1 negative remained This was in a 9 year-old girl who had previously been without antibodies for both Types 1 and 3 poliovirus; her Type 2 titer re-

Table 9 Cuba—Group 3—Matanzas City

Poliovirus Type 3 Antibody Response of 91 Persons Fed Oral Poliomyelitis Vaccine in 3 Feedings, 1 Week Apart

Distribution of Antibody Titers												Total Number Fed	No. with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			2		2	1	3	1	1			10	10
1:4							1		1			2	2
1:8					1		4					5	5
1:16							1	2	2			5	5
1:32					1		3	4	7	1	1	17	16
1:64								1	5		1	7	7
1:128							3	1	9	1	3	19	11
1:256							1	2		3	5	11	8
1:512								2	5		3	10	3
1:1024											3	3	
>1:1024										1	1	2	
Totals	—	—	2	—	4	1	16	15	30	6	17	91	69

Table 8 Cuba—Group 3—Matanzas City

Poliovirus Type 2 Antibody Response of 91 Persons Fed Oral Poliomyelitis Vaccine in 3 Feedings, 1 Week Apart

Distribution of Antibody Titers												Total Number Fed	No. with 4-Fold or Greater Response
Pre-Feeding	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4		1			1				1			3	3
1:4													
1:8											1	1	1
1:16													
1:32					1	1	2					4	2
1:64							2		1		1	4	2
1:128							3	5	5		1	14	6
1:256							1	1	3		3	8	3
1:512								1	13	4	6	24	6
1:1024									2	2	3	7	
>1:1024									1	2	23	26	
Totals		1			2	1	8	7	26	8	38	91	23

TABLE 10. CUBA—GROUP 4—SALK VACCINATION EFFECT
DISTRIBUTION OF 105 CHILDREN, BY AGE AND HISTORY OF PREVIOUS SALK VACCINATION

YEARS OF AGE	ORALLY VACCINATED CHILDREN					CONTROLS*
	TOTAL	NO. OF SALK INJECTIONS				
		NONE	ONE	TWO	THREE	
4	1	1				1
5	3				3	6
6	21	5		1	15	9
7	30	6		6	18	1
8	19	7		2	10	1
9	8			1	7	
10	5	1			4	
Total	87	20	—	10	57	18

* All controls had received 3 Salk injections

vaccinated contacts at the time the other children were given the oral poliovirus vaccine

Capsules containing 10 ° TCD_{50} of Type 2 virus were administered 27 Novmber 1958, and on 17 December $10^{5.5}$ TCD_{50} of Type 3 virus was fed The final capsule representing $10^{5.5}$ TCD_{50} of Type 1 polio virus was ingested 20 January 1959 Approximately 100 cc. of the resuspended dry fat free milk solids at pH 6 7 were given with each feeding Blood samples were collected from the vaccinated and control children at the time of the first feeding, and again 34 days after the last strain was fed

At the time the feeding program started there were 15 seronegative children given oral vaccine These were distributed as indicated in Table 11 In addition, there was a single negative (Type 3) among the control children who had received *three Salk* vaccine injections (Table 11)

Examination of the post vaccination serum specimens revealed five residual negatives in the orally vaccinated children a conversion rate of 67 per cent The Type 3 negative control child was still negative and still unchanged in titer for the other two strains As shown in Table 12 four of the five post-vaccination negatives were for Type 2 virus and three of these were in children who had had no Salk injections While only ten of fifteen negatives converted, *there were many booster responses among those* whose pre feeding antibody levels were from 1 4 and 1 512 These responses are summarized by sub-groups and virus type in Table 13 Here it may also be seen that only one of the control children who had been in contact with the orally vaccinated children, but had not received the live virus vaccine manifested a 4-fold increase in titer for Type 2 virus Distributed among the other 17 unvaccinated controls there were nine instances of a 2-fold titer increase, 13 of a 2 fold *titer decrease and four in which there were 4-fold* titer decreases. (Tables 13, 14, 15, 16, and 17)

The composite titer changes for sub-groups are illustrated by the geometric titers before and after the feeding program was started.

mained unchanged at 512. In addition to the 22 negatives which were converted, among children whose pre-vaccination titers ranged from 1 4 to 1:512, 21 of 71 children showed booster responses for Type 1 virus; twenty of 55 for Type 2, and 59 of 76 for Type 3 The magnitude of the collective response is suggested by the geometric mean antibody titers before and after feeding the three virus types at 7-day intervals.

GEOMETRIC MEAN ANTIBODY TITERS

Poliovirus Type	*Before*	*After*	*Fold Gain*
1	72	159	2 2x
2	371	709	1 9x
3	56	382	6 8x

Group IV

In a foundling home near the center of Havana where the majority of children had received two or three doses of Salk vaccine, an opportunity was afforded to observe the response of children to oral vaccination after artificial immunization It is not to be presumed that their pre-vaccination antibodies were entirely the result of the Salk vaccine The group of 105 children included 20 who had had no Salk vaccine and 10 who had received two vaccine injections, the second being given in April 1957. There were also 75 children who had completed their third Salk injection in May 1957, approximately 18 months prior to the initiation of the oral vaccination program on 27 November 1958

Approximately 80 per cent of the children had been resident in the institution for several years Their ages varied from 4 to 10 years and the median was 7 (Table 10) One child was an epileptic Fifty-six were white. There were 95 males A physician was in residence at the home

Eighteen children who had received three Salk vaccine injections and who were randomly located in dormitory quarters, were left as un-

Feedings One week apart Ages of Subjects 6 yrs —8 8 yrs —16 10 yrs —18
No of Subjects 91 7 yrs —24 9 yrs —16 11 yrs — 9

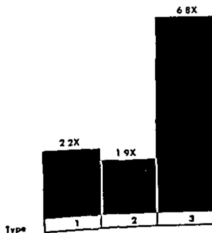
Antibody Titers
Geometric Mean by Type

	1	2	3
Pre-vaccination	72	371	56
Post-vaccination	159	709	382

Antibody Negatives

Total number	23
No responding	22
Percent responding	-96%

Fold Gain in Geometric Mean



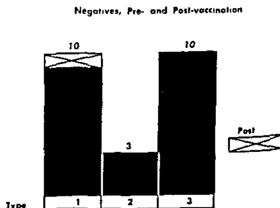


FIG 3 *Oral Poliomyelitis Vaccine* Cuba Group 3 - Matanzas City

TABLE 10 CUBA—GROUP 4—SALK VACCINATION EFFECT
DISTRIBUTION OF 105 CHILDREN, BY AGE AND HISTORY OF PREVIOUS SALK VACCINATION

YEARS OF AGE	ORALLY VACCINATED CHILDREN					CONTROLS*
	TOTAL	NO OF SALK INJECTIONS				
		NONE	ONE	TWO	THREE	
4	1	1				1
5	3				3	6
6	21	5		1	15	9
7	30	6		6	18	1
8	19	7		2	10	1
9	8			1	7	
10	5	1			4	
Total	87	20	—	10	57	18

* All controls had received 3 Salk injections

vaccinated contacts at the time the other children were given the oral poliovirus vaccine.

Capsules containing $10^{5.0}$ TCD_{50} of Type 2 virus were administered 27 Novmber 1958, and on 17 December $10^{5.8}$ TCD_{50} of Type 3 virus was fed. The final capsule representing $10^{7.5}$ TCD_{50} of Type 1 polio virus was ingested 20 January 1959 Approximately 100 cc of the resuspended dry fat free milk solids at pH 6 7 were given with each feeding Blood samples were collected from the vaccinated and control children at the time of the first feeding, and again 34 days after the last strain was fed

At the time the feeding program started there were 15 seronegative children given oral vaccine. These were distributed as indicated in Table 11 In addition, there was a single negative (Type 3) among the control children who had received three Salk vaccine injections (Table 11)

Examination of the post-vaccination serum specimens revealed five residual negatives in the orally vaccinated children, a conversion rate of 67 per cent. The Type 3 negative control child was still negative and still unchanged in titer for the other two strains As shown in Table 12, four of the five post-vaccination negatives were for Type 2 virus and three of these were in children who had had no Salk injections While only ten of fifteen negatives converted, there were many booster responses among those whose pre-feeding antibody levels were from 1 4 and 1.512 These responses are summarized by sub-groups and virus type in Table 13 Here it may also be seen that only one of the control children who had been in contact with the orally vaccinated children, but had not received the live virus vaccine, manifested a 4-fold increase in titer for Type 2 virus Distributed among the other 17 unvaccinated controls there were nine instances of a 2 fold titer increase, 13 of a 2 fold titer decrease and four in which there were 4-fold titer decreases (Tables 13, 14, 15, 16, and 17.)

The composite titer changes for sub-groups are illustrated by the geometric titers before and after the feeding program was started

mained unchanged at 512. In addition to the 22 negatives which were converted, among children whose pre-vaccination titers ranged from 1:4 to 1:512, 21 of 71 children showed booster responses for Type 1 virus, twenty of 55 for Type 2, and 59 of 76 for Type 3. The magnitude of the collective response is suggested by the geometric mean antibody titers before and after feeding the three virus types at 7-day intervals.

GEOMETRIC MEAN ANTIBODY TITERS

Poliovirus Type	*Before*	*After*	*Fold Gain*
1	72	159	2.2x
2	371	709	1.9x
3	56	382	6.8x

Group IV

In a foundling home near the center of Havana where the majority of children had received two or three doses of Salk vaccine, an opportunity was afforded to observe the response of children to oral vaccination after artificial immunization. It is not to be presumed that their pre-vaccination antibodies were entirely the result of the Salk vaccine. The group of 105 children included 20 who had had no Salk vaccine and 10 who had received two vaccine injections, the second being given in April 1957. There were also 75 children who had completed their third Salk injection in May 1957, approximately 18 months prior to the initiation of the oral vaccination program on 27 November 1958.

Approximately 80 per cent of the children had been resident in the institution for several years. Their ages varied from 4 to 10 years and the median was 7. (Table 10.) One child was an epileptic. Fifty-six were white. There were 95 males. A physician was in residence at the home.

Eighteen children who had received three Salk vaccine injections and who were randomly located in dormitory quarters, were left as un-

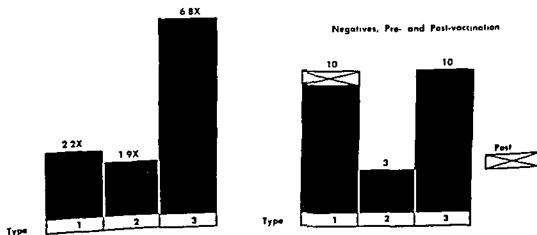


FIG. 3. Oral Poliomyelitis Vaccine. Cuba—Group 3—Matanzas City

TABLE 13 CUBA—GROUP 4—SALK VACCINATION EFFECT

GEOMETRIC MEAN ANTIBODY TITERS OF 87 CHILDREN BEFORE AND AFTER FEEDING OF ORAL POLIOMYELITIS VACCINE, BY *HISTORY OF* PREVIOUS SALK VACCINATION, AND OF 18 CONTROLS

History of Salk Injections	Number of Children	Geometric Mean Antibody Titers								
		Type 1			Type 2			Type 3		
		Pre-Feeding	Post-Feeding	Fold Gain	Pre-Feeding	Post-Feeding	Fold Gain	Pre-Feeding	Post-Feeding	Fold Gain
None	20	54	104	1.6x	79	187	2.4x	24	132	5.5x
Two	10	157	294	1.9x	415	446	1.1x	79	208	2.1x
Three	57	229	360	1.5x	496	711	1.4x	109	288	2.6x
Controls*	18	240	237		438	406		114	87	

* All controls had received 3 Salk injections.

TABLE 11 CUBA—GROUP 4—SALK VACCINATION EFFECT
DISTRIBUTION OF SERONEGATIVES AMONG 87 CHILDREN BEFORE FEEDING OF ORAL POLIOMYELITIS VACCINE

Poliovirus Type			Total Number	
1	2	3	Children	Negatives
X			1	1
	X		4	4
		X	4	4
X	X		1	2
X		X	1	2
	X	X	1	2
		Total	12	15

TABLE 12 CUBA—GROUP 4—SALK VACCINATION EFFECT
DISTRIBUTION OF SERONEGATIVES, BEFORE AND AFTER FEEDING OF ORAL POLIOMYELITIS VACCINE, BY HISTORY OF PREVIOUS SALK VACCINATION

History of Salk Injections		Type 1		Type 2		Type 3	
		Pre-Feeding	Post-Feeding	Pre-Feeding	Post-Feeding	Pre-Feeding	Post-Feeding
None		2	0	4	3	4	0
Two		1	0	1	1	1	1
Three		0	0	1	0	1	0
Totals	Pre-	3		6		6	
	Post-		0		4		1

67% Negatives Responded

Table 15. Cuba—Group I—Salk Vaccination Effect
Poliovirus Type 1 Antibody Response of 57 Persons with 3 Previous Injections of Salk Vaccine Fed Oral Poliomyelitis Vaccine

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding <1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024	Total No Fed	No With 4-Fold or Greater Response
<1:4												—	—
1:4												—	—
1:8									1			1	1
1:16												—	—
1:32							2					2	2
1:64						2	3				2	7	2
1:128						1	6	2	4		2	15	6
1:256							2	2	4		1	9	1
1:512							2	1	12	1	2	18	2
1:1024									1	1		2	
>1:1024										1	2	3	
Totals	—	–	—		–	3	15	5	22	3	9	54	14

TABLE 14 CUBA—GROUP 4—SALK VACCINATION EFFECT

Poliovirus Antibody Gains of 4-Fold or Greater Following Oral Vaccination, Among Children with Pre-Vaccination Titers Between 1:4 and 1:512, by Virus Type and History of Previous Salk Vaccination

HISTORY OF SALK INJECTIONS	POLIOVIRUS TYPE		
	1	2	3
None	9/19*	7/15	12/20
Two	4/7	0/5	5/9
Three	14/52	11/37	23/54
Controls†	0/18	1/15	0/17

* Number of 4 fold or greater responses, number with pre-vaccination titer between 1 4 and 1 512
† *All controls had received 3 Salk injections*

DISCUSSION

In addition to the four groups of children vaccinated with the oral poliovirus vaccine described above, we have vaccinated ten other groups during the past year The 14 groups comprised approximately 2,000 children In none of these have nervous symptoms or other disturbances been observed which were suspected of association with the administration of the vaccines. Not only was the clinical course of the normal children entirely uneventful while undergoing immunization, but so also was that of many

[illegible]

there were no adverse reactions in one epileptic,

[illegible]

seen in undernourished, parasitized children when brought together in recreation camps and institutions, but these illnesses were no more numerous and no more severe than those observed prior to the initiation of the oral poliovirus vaccination.

We believe that it is important to record that the infectious diseases mentioned above were not aggravated in any way by their concurrence with an induced infection with attenuated strains of poliovirus. Conversely, it is equally important to find that these induced infections are not altered in any clinically discernible manner by the presence of certain common diseases of childhood which are likely to occur during an immunization program.

Since the serologic studies on a large proportion of these 2,000 children are not yet completed, it remains to be determined whether or not concurrence of these fortuitous acute infections have adversely affected the establishment of the vaccine strain in the gastro-intestinal tract A number of vaccinated children were undergoing atabrine treatment for infestations with *Giardia lamblia* and this may have adversely affected the outcome of the oral vaccination Completion of the serologic tests on the sera of these individuals should provide answers to these important questions

It should be emphasized also that several of these small-scale trials were conducted under conditions that were not likely to be favorable to the vaccines This was done because it was thought desirable, as a guide in planning future and larger scale programs, to know or to have some indication as to what might be expected when administrators encounter the numerous conditions and complications that are certain to develop with expanding use of the vaccine under all conditions. Our experiences to date have been most reassuring and, therefore, we plan to continue our studies and to seek evidence of circumstances and conditions both medical and environmental which may contraindicate the use of the vaccines

Though small in scope and number of subjects, the first trial provided an indication that common carbonated beverages having a high degree of acidity were not markedly inhibitory when consumed immediately after the ingestion of the vaccine strains in capsule form The second trial group was large enough to give clear evidence of the fact that the simultaneous administration of all three strains can be a practical, effective procedure This fact, now confirmed by other larger trials, is of great practical importance. The third trial, contrary to expectation, implies that interference between these three

TABLE 15. CUBA—GROUP 4—SALK VACCINATION EFFECT
POLIOVIRUS TYPE 1 ANTIBODY RESPONSE OF 57 PERSONS WITH 3 PREVIOUS INJECTIONS OF SALK VACCINE FED ORAL POLIOMYELITIS VACCINE

Pre-Feeding	Distribution of Antibody Titers — Post-Feeding											Total No Fed	No With 4-Fold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4												—	—
1:4												—	—
1:8									1			1	1
1:16												—	—
1:32							2					2	2
1:64						2	3				2	7	2
1:128						1	6	2	4		2	15	6
1:256							2	2	4		1	9	1
1:512							2	1	12	1	2	18	2
1:1024									1	1		2	
>1:1024										1	2	3	
Totals	—	—	—	—		3	15	5	22	3	9	54	14

TABLE 14 CUBA—GROUP 4—SALK VACCINATION EFFECT

Poliovirus Antibody Gains of 4-Fold or Greater Following Oral Vaccination, Among Children with Pre-Vaccination Titers Between 1:4 and 1:512, by Virus Type and History of Previous Salk Vaccination

HISTORY OF SALK INJECTIONS	POLIOVIRUS TYPE		
	1	2	3
None	9/19*	7/15	12/20
Two	4/7	0/5	5/9
Three	14/52	11/37	23/54
Controls†	0/18	1/15	0/17

* Number of 4-fold or greater responses/number with pre-vaccination titer between 1:4 and 1:512
† All controls had received 3 Salk injections

DISCUSSION

In addition to the four groups of children vaccinated with the oral poliovirus vaccine described above, we have vaccinated ten other groups during the past year. The 14 groups comprised approximately 2,000 children. In none of these have nervous symptoms or other disturbances been observed which were suspected of association with the administration of the vaccines. Not only was the clinical course of the normal children entirely uneventful while undergoing immunization, but so also was that of many children who developed acute infectious diseases, such as mumps, rubella, or influenza while participating in the oral vaccination trials. Similarly there were no adverse reactions in one epileptic, one diabetic, and several children suffering from cardiac and kidney ailments. There were numerous minor illnesses, such as are invariably seen in undernourished, parasitized children when brought together in recreation camps and institutions, but these illnesses were no more numerous and no more severe than those observed prior to the initiation of the oral poliovirus vaccination.

We believe that it is important to record that the infectious diseases mentioned above were not aggravated in any way by their concurrence with an induced infection with attenuated strains of poliovirus. Conversely, it is equally important to find that these induced infections are not altered in any clinically discernible manner by the presence of certain common diseases of childhood which are likely to occur during an immunization program.

Since the serologic studies on a large proportion of these 2,000 children are not yet completed, it remains to be determined whether or not concurrence of these fortuitous acute infections have adversely affected the establishment of the vaccine strain in the gastro-intestinal tract. A number of vaccinated children were undergoing atabrine treatment for infestations with *Giardia lamblia* and this may have adversely affected the outcome of the oral vaccination. Completion of the serologic tests on the sera of these individuals should provide answers to these important questions.

It should be emphasized also that several of these small-scale trials were conducted under conditions that were not likely to be favorable to the vaccines. This was done because it was thought desirable, as a guide in planning future and larger scale programs, to know or to have some indication as to what might be expected when administrators encounter the numerous conditions and complications that are certain to develop with expanding use of the vaccine under all conditions. Our experiences to date have been most reassuring and, therefore, we plan to continue our studies and to seek evidence of circumstances and conditions, both medical and environmental, which may contraindicate the use of the vaccines.

Though small in scope and number of subjects, the first trial provided an indication that common carbonated beverages having a high degree of acidity were not markedly inhibitory when consumed immediately after the ingestion of the vaccine strains in capsule form. The second trial group was large enough to give clear evidence of the fact that the simultaneous administration of all three strains can be a practical, effective procedure. This fact, now confirmed by other larger trials, is of great practical importance. The third trial, contrary to expectation, implies that interference between these three

TABLE 17. CUBA—GROUP 4—SALK VACCINATION EFFECT
POLIOVIRUS TYPE 3 ANTIBODY RESPONSE OF 57 PERSONS WITH 3 PREVIOUS INJECTIONS OF SALK VACCINE FED ORAL POLIOMYELITIS VACCINE

	Distribution of Antibody Titers											Total No Fed	No. With 4-Fold or Greater Response
	Post-Feeding												
Pre-Feeding	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			1									1	1
1:4												—	
1:8							1					1	1
1:16			1		1			1				3	1
1:32					1	1	2	1	2			7	5
1:64							3	1	4	1		9	6
1:128							8	1	6	1	1	17	8
1:256								3	6	1		10	1
1:512									4	2	1	7	1
1:1024									1		1	2	
>1:1024												—	
Totals	—	—	2	—	2	1	14	7	23	5	3	57	21

Table 16. Cuba—Group 4—Salk Vaccination Effect
Poliovirus Type 2 Antibody Response of 57 Persons with 3 Previous Injections of Salk Vaccine Fed Oral *Poliomyelitis Vaccine*

Pre-Feeding	Distribution of Antibody Titers: Post-Feeding <1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024	Total No Fed	No With 4-Fold or Greater Response
<1:4			1									1	1
1:4												—	—
1:8												—	—
1:16					1							1	0
1:32												—	—
1:64												—	—
1:128								2	4			6	4
1:256							1	2	1	1	1	6	2
1:512							1		15	4	4	24	4
1:1024									2		3	5	
>1:1024									1	2	11	14	
Totals	—	—	1	—	1	—	2	4	23	7	19	57	11

TABLE 17 CUBA—GROUP 4—SALK VACCINATION EFFECT
POLIOVIRUS TYPE 3 ANTIBODY RESPONSE OF 57 PERSONS WITH 3 PREVIOUS INJECTIONS OF SALK VACCINE FED ORAL POLIOMYELITIS VACCINE

Pre-Feeding	Distribution of Antibody Titers											Total No Fed	No With 4-Fold or Greater Response
	Post-Feeding												
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4			1									1	1
1:4												—	
1:8							1					1	1
1:16			1		1			1				3	1
1:32					1	1	2	1	2			7	5
1:64							1	1	4	1		9	6
1:128							8	1	6	1	1	17	8
1:256								1	6	1		10	1
1:512									4	2	1	7	1
1:1024									1		1	2	
>1:1024												—	
Totals	—	—	2	—	2	1	14	7	23	5	3	57	21

strains of poliovirus is not a major factor when given at intervals of seven days in the order Types 2, 3, 1, since 22 of the 23 negatives converted and many of the 62 children showed good booster responses to this system of administration

While the fourth trial provided no substantial evidence of contact spread of the vaccine strain under our test conditions, the relative titer stability of the controls during the trial period of approximately 12 weeks underscores the significance of titer changes yielded by the assay method. The number and magnitude of the responses in the vaccinated children were not remarkable, in part perhaps because of the relatively high pre-vaccination titers

Viewing the four trial groups described above as a whole, there were 256 children, ranging in age from 4 to 17 years who were fed all three types of virus and from whom pre- and post-vaccination sera samples were assayed for antibodies Most of the children were between 6 and 10 years of age and they represented a variety of social and economic conditions which are typical of those to be encountered in Cuba and many other Latin American countries The results obtained in terms of responses to the three strains of poliovirus have been somewhat variable, but so have the test conditions Some of the groups have been too small to give clear-cut answers but they have been useful in spite of this limitation An inherent invariable in all such studies is the degree of pre-existing immunity for each type of virus as indicated by antibody levels Our subjects were selected on the basis of availability rather than whether or not they represented optimum media for obtaining results that would be most favorable to the vaccines For this reason, we believe that we are observing the performance of the oral vaccines in a reasonable cross-section of conditions as they exist in the field

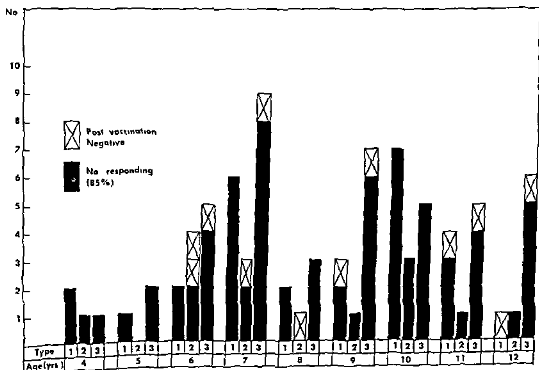


Fig 4 Havana, Cuba, Combined Data on Distribution of Poliomyelitis Seronegatives among 256 Children, by Age and Virus Type, Before and After Oral Vaccination with Poliovirus Vaccine

TABLE 18 COMBINED DATA—HAVANA, CUBA
DISTRIBUTION OF POLIOVIRUS SERONEGATIVES, BEFORE AND AFTER ORAL POLIOMYELITIS VACCINATION

Group	Total Subjects in Group	Pre-Vaccination				Post-Vaccination			
		Virus Type			Total Negatives	Virus Type			Total Negatives
		1	2	3		1	2	3	
No 1	16	4	2	6	12	0	0	2	2
No 2	62	11	4	21	36	2	0	2	4
No 3	91	10	3	10	23	1	0	0	1
No 4	87	3	6	6	15	0	4	1	5
Total	256	28	15	43	86	3	4	5	12

In assessing the value of the vaccine there are several criteria that may be applied First, of course, is that of safety, and this we have already discussed Second is the reduction of the hazard of paralytic poliomyelitis by the elimination of susceptibles (Fig 4) Figure 4 shows that the conversion rate was approximately 85 per cent in the 4 trial groups The few failures have been scattered over the whole age range investigated and they have not been confined to a single type of virus (Table 18)

In individual trials the conversion rates ranged from 67 to 96 per cent When the three strains of virus were fed simultaneously the rate was 89 per cent Associated with the reduction of the number of susceptibles is the frequency of booster responses Directing attention to those children whose pre vaccination titers varied from 1 4 to 1 512, because the titrations were not done at dilutions high enough to detect 4-fold increases at levels higher than 512 33 per cent of 210 children had booster responses for Type 1 virus, 43 per cent of 173 for Type 2 and 58 per cent of 208 for Type 3 The mean titers (Table 19) show clearly that the least gains were made in groups which had the highest antibody levels at the time of feeding Gains for Type 2 virus in general were less marked than for the other two types of virus Fortunately, this is the type which appears to be associated with epidemics least often and further work in the selection of strains will doubtlessly provide a blend of more equally balanced strains for the oral vaccine We believe that the collective data argue effectively for the efficiency of the present strains and the method of application

Another criterion that may be applied in evaluating the results of our trials is that of practicality and utility of the method of application. Here there would appear to be a strong argument in its favor With the increasing evidence of the high response rates to the simultaneous feeding of all three strains of virus vaccination against poliomyelitis is transformed from a clinical procedure into a beautifully simple tool for mass application It lends itself to emergency conditions as well as to the less urgent demands of routine prophylaxis in the clinic, the office or institution From the viewpoint of speed and ease of application, the trivalent oral vaccination against poliomyelitis may be favorably compared to the mass dusting with insecticides in control of epidemic typhus And from the standpoint of cost, which is always an important consideration for the public health administrator, trivalent oral vaccination should certainly make smaller demands on our resources.

TABLE 19 COMBINED DATA—HAVANA, CUBA
GEOMETRIC MEAN POLIOVIRUS ANTIBODY TITERS BEFORE AND AFTER ORAL POLIOMYELITIS VACCINATION

Group	No of Children in Group	Type 1			Type 2			Type 3		
		Titer		Fold Gain	Titer		Fold Gain	Titer		Fold Gain
		Pre-	Post-		Pre-	Post-		Pre-	Post-	
No 1	16	33	111	3 4x	128	412	3 2x	15	38	2 5x
No 2	62	35	126	3 6x	162	462	2 8x	13	115	8 8x
No 3	91	72	159	2 2x	371	709	1 9x	56	382	6 8x
No 4 No Salk	20	54	194	3 6x	79	187	2 4x	24	132	5 5x
Two Salk	10	157	294	1 9x	415	446	1 1x	79	208	2 1x
Three Salk	57	229	386	1 5x	496	711	1 4x	109	288	2 6x
Combined	256	76	169	2 2x	272	519	1 9x	40	209	5 2x
Controls	18	246	237		438	406		114	87	

SUMMARY

The serological and clinical responses of 256 children who were given the three serotypes of attenuated poliovirus as an oral vaccine are described and reference is made to the clinical observations made during 10 additional trials comprising in all approximately 2,000 children ranging in age from 1 to 21 years The children represent a variety of field conditions in Cuba

Clinical evidences of nervous or other disturbances attributable to the vaccine were not observed, and vaccination in the presence of acute infectious diseases such as mumps, rubella, and influenza as well as in certain chronic diseases was uneventful

Conversion rates for seronegatives to positives varied for the three types of poliovirus and in the four trial groups, the over-all rate for the study was 85 per cent In addition, booster responses were shown by children whose pre-feeding titers were between 1:4 and 1.512 The response rates in such children were 33 per cent for Type 1 poliovirus, 43 per cent for Type 2 and 58 per cent for Type 3.

The high rates of response when all three strains of poliovirus were administered simultaneously is a finding of considerable practical importance and its implications are discussed

We gratefully acknowledge the cooperation of Drs. Carlos Hernández Miyares, Alvaro Silva, María Oliva Barreiro, and Daniel Alonso, who clinically followed up the cases corresponding to the different groups included in this study

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DISCUSSION

CHAIRMAN ARMSTRONG The paper presented by Dr Embil is now open for discussion

DR GELFAND I would like to make a comment and a general suggestion This study, as well as many of those previously presented, has reported in detail on the antibody titer rises that occurred in individuals with pre-existing antibody

The significance of reinfection is uncertain, but it certainly is inconsequential compared to the significance of primary infection, whether one is interested in effectiveness or safety

Moreover, this study and many of the previous ones have reported the geometric mean titers of groups of people before and after vaccination

Reporting geometric mean titers combines the unimportant data on reinfections with the important data on primary infection, furthermore, it actually prejudices the demonstration of a vaccine effect, because even with demonstrated reinfection, individuals who start with high titers are much less likely to manifest titer rises when they have been reinfected

Therefore, the effect of reporting mean titer is to bias the result against the demonstration of vaccine effectiveness that actually was present

I should therefore like to suggest that in future field studies, since the phenomenon of reinfection accompanying primary infections has been adequately demonstrated, field laboratories save themselves all of the tremendous effort of studying post vaccination titer rises and concentrate almost exclusively on primary infections

This, of course, would not apply to those special studies that are specifically oriented toward the phenomenon of reinfection, but would apply to the majority of field investigations

DR. COX: I concur in Dr. Gelfand's remarks with regard to the geometric mean method of calculation, but I would also like to remind Dr. Gelfand that in the manuscript which he has the tables are broken down on each trial by sero-type and by individuals, so if there is any doubt in his mind as to what happened, it is just a matter of minutes until we can find out what did happen.

If we had been guilty of letting Dr Embil present this on geometric mean titers alone, then I would feel very badly.

DR BARR· I was waiting for the advocates of interference to say something about this, because I did not see any in these results

DR COX. As we stand now thus far, I think that probably the worst conditions that we could have asked for in trying to find interference would have been in the trial with the vaccines that were fed at seven-day intervals, because certainly by then we would expect to see the virus established in the gut at its maximum quantity

As stated in Dr Embil's paper, every one of us were quite surprised to find that the results obtained by the seven-day interval feeding, as far as could be seen on these small numbers, were just as good as though they had been fed at 21-day intervals or simultaneously

Furthermore, as we indicated yesterday, we have seen no evidence in our work carried out in Latin America that other enteric viruses do interfere Now, perhaps Mexico is an unusual country in this respect, but we certainly have not seen it in Colombia and Cuba; and I cannot believe that enteric virus infections are limited to Mexico

DR SABIN· Certain things are not necessary to repeat, because they have been said again and again and again, and I think they have been amply demonstrated

I think any attempt to determine interference by feeding virus in conditions such as this, and then testing at a certain interval later, is no way to measure it When I say conditions such as this, I have reference to conditions either in the home, on the street, or in an institution where the possibility for reinfection by picking up the virus that is going around there is very real

As I pointed out in the first paper that I pre-

sented on this program, the way to find out what is going on is under conditions where you study each individual child, you measure the viruses that are excreted, you determine what is there you see when infection has occurred, when antibody has developed, and when it has disappeared; then you know

I am quite sure that neither Mexico nor Africa nor the other Latin American countries, nor the United States, where I have studied this under certain conditions, are any different. These are quantitative aspects

From the data presented on the studies in Latin America, my conclusion is that there is ample evidence for interference But that is not the way to demonstrate it

Thus, with the strains that we have studied, under individual specified conditions, we cannot show the operation of interference Dr Chumakov, Dr Smorodintsev, in studies in institutions administering all three types, or in studies of random groups, where the opportunities for reinfection were there, obtained the same results They say they cannot see interference

I think that the conclusion is that under certain conditions of extensive spread the effect of interference is greatly minimized after a time but you cannot use such data to say there is no interference The phenomenon of interference must be understood in relation to group effects and in relation to individual effects

Dr Gelfand I would like to add one thing to what Dr Sabin has said

In any one place the enteric infections that are predominant are not constant They come and go in waves, so that a given field study in one place at one time which shows interference might not show it if done at a different time, and, conversely, when interference is not demonstrated, perhaps it might have been demonstrated at a different time

Therefore, in addition to the recirculation of the viruses that are passing from person to person, we have to consider the biology of the potentially interfering viruses

Dr Barr No one has said there is no interference, I would remind Dr Sabin

My only opinion is that interference has been overemphasized in this session, on the basis that it may occur in large numbers of cases I am quite certain, from what we have seen, that dosage and a number of other things will overcome it or have a real tendency to do so

Dr Sabin I am very anxious to arrive at spheres of agreement, and I think that when we talk long enough we can find them

I want to agree with Dr Barr that interference must be understood, but not overemphasized. It must not be stressed to the point where someone will say "Well, you cannot do anything about it, because there is interference" I think that our problem now is to find the best way to overcome it And I think that it can be overcome, without any question of a doubt

Among the things that we have discussed is the question of providing opportunities for sufficient spread, for introducing massive amounts of the virus so that we will not have 10 soldiers trying to interfere with 10 000 but at least try to match 10 000 with 10 000 I am sure that all those problems can be overcome Dr Barr I think we can agree

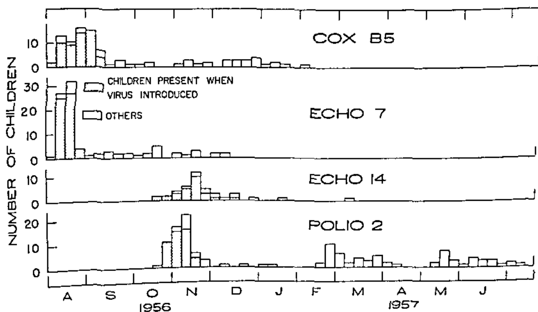
Chairman Armstrong Dr Bell

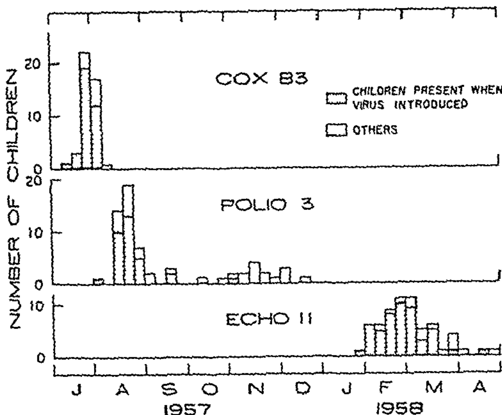
Dr Bell The question of virus interference has been an important one at this Conference and we at the Laboratory of Infectious Diseases of the National Institutes of Health, have some data from a very intensive five-year study of institutionalized children that suggest caution in interpretation of data on virus interference The children were babies 6 to 35 months of age The mean weekly population was about 50 but an average of 4 new babies are admitted each week From each child, we routinely collected both anal and oral specimens for virus and bacteriologic study daily for the first 10 days after admission once a week thereafter, and at onset of each illness You can appreciate that this represents a lot of specimens—some 4,000 to be tested each month

I would like to show you the occurrence of some enteric virus infections in this group Slide 1 covers the period 1955 and 1956

It can be seen that we first had an outbreak of infection with ECHO 8, a few weeks later with ECHO 13, a few weeks later, with ECHO 12, a few weeks later, with ECHO 14, and then a few weeks later, with ECHO 20 The bars represent children only at the time when first in-

NUMBER OF CHILDREN
20
10
0
ECHO 8
10
0
ECHO 13
10
0
ECHO 12
10
0
ECHO 14
CHILDREN PRESENT WHEN VIRUS INTRODUCED
OTHERS
20
10
0
ECHO 20
S O N D J F M A M J J
1955 1956





fection was *recognized*—the continued shedding of the virus in a child *is not* shown The shaded bars represent children who were *in residence* at the time the first child in the group was *found* infected; the clear bars represent children admitted later You can see the *initial* rapid spread of infection among children present in the group, and then the infections occurring among new children as they were constantly being admitted over a period of many months

Slide 2 shows the 1956 and 1957 experience, and here we find Coxsackie B5 and ECHO 7, both occurred at *practically the same time*, with apparently no interference between outbreaks of these two viruses Then we have a *second* outbreak of ECHO 14 occurring practically at the same time as *an* outbreak of polio Type 2

Slide 3 shows the 1957 and 1958 experience For some unknown reason, new infections with Coxsackie B3 did not continue to occur among new admissions, but a polio Type 3 outbreak immediately occurred These are only the enteric virus infections, the highly prevalent adenoviruses, and the *many* outbreaks of bacterial infections have not yet been tabulated in a similar manner

From these slides, *one* might *off hand* conclude that the *sequence* of virus *infection* outbreaks suggests virus interference between some but not all, enteroviruses Now, quite aside from the difficulty of finding two viruses, even though present in the same tissue culture, the *important* thing is that in most of these outbreaks of infection the first child found infected was a newly-admitted child, positive in his admission specimen Hence, in this intensive study of many outbreaks of infection in a small group, *we have* great difficulty in concluding that there was virus interference in outbreaks I trouble you with these data only to inject a word of caution in deducting virus interference from more meager data of large groups Dr Leon Rosen of my laboratory did the laboratory work on enteroviruses and presented these data in Portugal last year

17. POLIOMYELITIS VACCINATION WITH SABIN ORAL VACCINE IN MEXICO CITY AND SIX PRECINCTS OF THE FEDERAL DISTRICT

DR. ALEJANDRO GUEVARA ROJAS
MINISTRY OF HEALTH AND WELFARE, MEXICO CITY, MEXICO

DR GUEVARA ROJAS. The program begun in Mexico City and the Federal District on 23 February of this year, for the purpose of administering 100,000 doses of Sabin oral vaccine in each of the 3 types, is now in its final phase

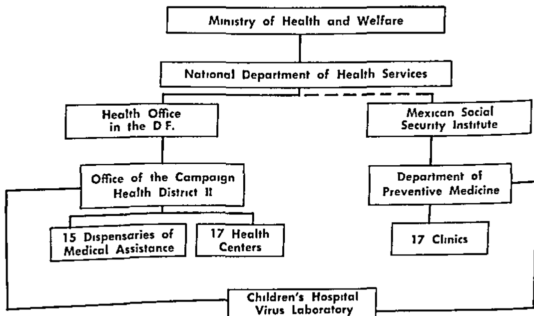
Hence the data given in this report are provisional and deal exclusively with the health administration aspects, since the epidemiological facts will be studied later, and the laboratory findings were dealt with by Ramos Alvarez

Background

In June 1956 the Health Department in the Federal District (D F.) ordered the use of the Salk poliomyelitis vaccine as a prophylactic measure to be taken in the health districts of the D F

Later, the Children's Hospital of Mexico City undertook, under the direction and responsibility of Ramos Alvarez, experimental vaccination whose satisfactory results were justification for the Department of Preventive Medicine of the Mexican Social Security Institute to study and plan the administration of the Sabin vaccination to the children of the insured, through the 17 clinics that function in Mexico City and in the Precincts of Atzcapotzalco, Ixtapalapa, Coyoacán, Magdalena Contreras, Tlalpan, and Villa Obregón, of the D F.

For the organization of this program, the persons to be vaccinated were limited to those between 6 months and 4 years of age, because the incidence of the disease in the Federal District is higher in that group; schedules were drawn up both for the program in general and for each administration stage of the three types of vaccine; instructions were given to the personnel on the technical and administrative aspects of the work,



forms for the documentation that would have to be used were prepared; and instructions were issued also for giving preparatory health education on vaccination to the insured

It should be stated that the Mexican Social Security Institute is a decentralized official agency, with medical functions

The program drawn up by the Institute and the Children's Hospital in Mexico City was presented in February of this year to the Secretary of Health and Welfare who, since all the facts were favorable, agreed to grant authorization for the work on a large scale and ordered the health centers in the areas coming under the jurisdiction of the Social Security clinics to participate in the program in the same period and in equal proportion

In this form, the work to be done would have two aspects:

(1) Vaccination of the controlled group among the insured under the Mexican Social Security Institute

(2) Vaccination of uncontrolled groups in the 17 health districts

Program

The work was organized as follows

The virus laboratory of the Children's Hospital would provide the vaccine, make the laboratory studies and, through the specialized medical services of the hospital, contribute to the clinical study of cases presenting problems

The technical and administrative direction of the work in the groups in the health districts was assigned to the undersigned, and that in the Social Security Clinics to the Chief of the Department of Preventive Medicine of the Institute Dr Rafael Alvarez Alva

The work was to be done in the 12 health districts of Mexico City, and in 6 of the D F (the health zoning coincides with the political division in wards and precincts), which would cover the following areas with their respective estimated populations,

Health Districts	Estimated Population, 1959
District I	603,604
District II	302,994
District III	387,326
District IV	200,207
District V	117 355
District VI	206,139
District VII	303,440
District VIII	302,436
District IX	489,635
District X	213,000
District XI	298,220
District XII	267,932–3,092 288
District of Atzcapotzalco	315,611
District of Ixtapalapa	120,723
District of Coyoacán	117 608
District of Magdalena Contreras	90,061
District of Tlalpan	56,086
Villa Obregón	106,535– 806,627

The health districts corresponding to the following precincts will be excluded from the program

Health District	Estimated Population, 1959
Gustavo A Madero	346,292
Ixtacalco	57,027
Tláhuac	32,770
Milpa Alta	30,596
Xochimilco	79,097
Cuajimalpa	16,295–562,077

On 18 February the authorities of the health districts were ordered to carry out the program, under the following general conditions.

1 The work was to begin 23 February 1959

2 The personnel to be used was the regular staff assigned to each health center

3 The vaccinations should be given without interfering with the normal activities of each dependency

4 In the period from 23 February to 4 April 50,000 applications of Sabin oral vaccine Type 1, should be given

5 The vaccination would be wholly voluntary, both for the children registered in the health

centers, and for those of the rest of the population.

6 To ensure the conclusion of the series, each district would accept persons resident in the area

7 The vaccinations could be concluded with Salk vaccine for those who so requested

8 Poliomyelitis vaccinations would be begun with Sabin live virus vaccine only

9 The information on the vaccine would be given to the public in the 4 days prior to the initiation and during the course of the program, by means of direct information by the personnel of the health centers, especially the visiting nurses in their house visits, *written publicity in the press, information* and publicity by radio and television (These conditions were reported by memoranda from the Health Office in the D F)

10 The documentation for the compilation and centralization of data, reports, appointments, etc , would be the same or similar in the work of the health districts and the social security clinics, and would consist of

- (a) Individual card for alphabetical record
- (b) Set of individual cards with an original and two copies (each one of a different color) with a numerical index, for registration of data
- (c) Daily activities record sheet
- (d) Daily absentee record sheet
- (e) Appointment-record postcard
- (f) *Directories of the health centers and of* the Mexican Social Security Institute clinics.
- (g) Calendar for applications of the vaccine
- (h) Sheet for numerical concentration of persons vaccinated, which modified the tables of the Mexican Social Security Institute, classifying the persons vaccinated by age and sex It was used also for periodic concentrations

11 To guarantee optimum conditions for the work (medical examination, preservation of the vaccine, and compilation of data), the vaccine was to be administered in selected establishments only.

Development of the program:

It was arranged with the Virus Laboratory of the Children's Hospital that the supplies of vaccine would be delivered weekly after proof of the previous delivery by means of the records of persons vaccinated The purpose of this measure was to prevent keeping diluted products under refrigeration for more than a week Delays and even temporary interruptions in the work occurred, for lack of the product, but Ramos Alvarez should be credited with understanding the administrative problems that occasionally caused delays in preparing and delivering reports

The personnel that was available at each health center varied in number, but in essence it was as follows:

Director of the health center, responsible for coordination, one epidemiologist, 1 to 5 pediatricians, 1 to 2 visiting physicians; 1 *health* educator; 1 to 8 visiting nurses, 1 or 2 nurses for vaccination; 2 administrative aides. A varying number of visiting nurses were added to each center, to check on the persons vaccinated

The materials used in administering the vaccine were:

Dropper bottles with calibrated dropper, to administer 0 1 ml of vaccine, per dose

Plastic dropper bottles for syrup

Cherry- or raspberry-flavored syrup

Storage bottles with a 2 per cent solution of hydrochloric acid

Axillary thermometers

Storage bottles with 95° alcohol for the thermometers

The method of administering the vaccine was as follows

On request, every child was given a medical examination by a pediatrician, to ascertain the state of health, the lack of fever being ascertained by a systematic taking of the axillary temperature Those who did not meet the appropriate health conditions were rejected or put off until later

The children who were qualified were given, in a perfectly clean plastic spoon, 0 1 ml of the vaccine solution kept under refrigeration, in a vehicle of 1 to 2 ml of cherry- or raspberry-flavored syrup, the total ingestion of the contents being checked

The used spoons were placed in a 2 per cent solution of hydrochloric acid for 12 hours, at least, and later were washed with distilled water before being used again

The individual records were kept on the al-

phabetical index card, and on the set of individual cards with the numerical index, copies of which were distributed thus: original (white) for the Central Bureau, to be returned at the end of the program; the first copy (yellow) for the applying office; second copy (blue) to be given to the person concerned, for whom it would serve as a record and vaccination certificate. Every endeavor was made to compile the data carefully.

Each health center or administering dependency prepared the reports of the day's activities and reported to the Central Office of the Program by telephone the number of persons vaccinated by age and sex.

The daily data were put together in weekly reports that were handed in in writing.

The Central Office of the Program summarized the data received in cumulative charts, by day and by office, and later prepared cumulative charts on the number of doses administered both for each dependency and by stage.

The National Poliomyelitis Campaign required that in a majority of the cases a check be made of the persons vaccinated, for the purpose of investigating any upsets that occurred in persons vaccinated and in contacts data which were entered on a special card, the agency also authorized the appointment of trained nurses with experience in similar work in supervision of Salk vaccine. The nurses were to receive 400 pesos a month for 3 hours a day of work (usually in the evening) for a period of 3 months. The manner in which they were assigned will be detailed later. This was the only actual extra expenditure incurred by the program.

The visiting nurses of the health centers were instructed to stress the vaccination program in their house visits, for the purpose of encouraging people to come to the centers, giving specific instructions on poliomyelitis vaccination and Sabin oral vaccination, and keeping a check on the children vaccinated and on their contacts.

It should be stated that during the entire program care was taken not to mislead the public by deprecating the Salk vaccine.

The first stage of vaccination was accomplished according to schedule in both the Mexican Social Security Institute and in the Health Office in the D.F., the only regrettable factor was that in the last few days the supply of vaccine fell short which made it impossible to reach the goal of 50,000 persons that had been set.

When the second stage was begun (administration of Type 3) there began to be irregularity in keeping appointments, and for this reason it was foreseen that this second phase would last some days longer than the estimated time, in order to make it possible to achieve the greatest percentage of complete treatments.

The situation was similar, although better, in the Social Security clinics, which had a high percentage of due attendance, while in the health centers it was necessary to use the procedures of inducing people to return by means of postal reminders, house visits by nurses and by medical personnel. In no case were more than 4 visits made.

Beginning in March, the incidence curves of measels whooping cough, enteritis and diseases of the respiratory tracts rose in Mexico City, and very often the administration of the second dose had to be postponed and even suspended for those reasons.

In view of the foregoing facts, instructions were prepared to cover those cases.

At present, the Mexican Social Security Institute has almost completed the third substage (administration of Type 2), and Dr Rafael Alvarez Alva, Chief of the Department of Preventive Medicine of the Institute, has reported that possibly the program will be completed in the latter part of June or the first days of July. Perhaps the health centers will be able to conclude their administrations at the same time. It will then be possible to make a recapitulation and final study, which will permit the preparation of a definitive over-all report.

Results

In Tables 1 and 2 are summarized the results obtained by the Mexican Social Security Institute clinics and the dependencies of the Ministry of Health and Welfare (health centers and dispensaries), with respect to those vaccinated with Type 1. They are listed and classified by age and sex, but the figures do not include 5,650 children who were vaccinated directly by the Children's Hospital.

In making the summary, consideration was given to the zone of influence of each of the Social Security Institute clinics, in order to add their data to the corresponding health district.

To repeat, the health zoning in Mexico City and in the Federal District coincides with the political division in wards and precincts, respectively.

It may be observed that the figure reached was higher than that which had been expected and the greater proportion of that figure should be credited to the Social Security clinics, although the difference under 5 per cent is explained by the following reasons:

1 Greater opportunity to make preparations for the work several months in advance
2 Timely census of the individuals of an age to receive the vaccine
3 Possibility of controlling the segment of the

TABLE 1 SUMMARY OF FIRST DOSES OF SALK ORAL VACCINE ADMINISTERED IN MEXICO CITY AND SIX PRECINCTS OF THE FEDERAL DISTRICT (FROM 23 FEBRUARY—4 APRIL 1959)

HEALTH DISTRICT	AGE					SEX		TOTAL
	6 TO 11 M	1 YEAR	2 YEARS	3 YEARS	4 YEARS	M	F	
I	1,797	2 137	2 237	2 299	3,638	6 097	6,011	12,108
II	1 477	1,872	1 818	1 781	2,410	4 790	4 568	9,358
III	2 504	2 856	2 785	2,736	3,808	7,347	7 342	14,689
IV	376	449	425	390	483	1 088	1,035	2,123
V	246	338	290	300	476	842	808	1,650
VI	562	742	808	779	1 063	1,996	1 958	3 954
VII	1 272	1 722	1 729	1 679	2 578	4 543	4 437	8 980
VIII	598	721	797	793	1 584	2,303	2 230	4 533
IX	1,731	2 051	1 935	2 090	3 317	5,678	5,446	11,124
X	970	1 332	1 214	1,226	1 787	3,221	3,308	6,529
XI	863	1 034	1 023	1 062	1 644	2 800	2 826	5 626
XII	793	1 076	1 059	1 070	1 133	2 598	2 533	5 131
Atz	612	778	823	841	1,235	2,137	2 152	4,289
Ixt	551	601	534	563	1 080	1 693	1 636	3,329
Coy	302	388	408	368	751	1 136	1 081	2 217
Cont	280	274	401	447	549	983	965	1 948
Tlalpan	170	250	289	327	556	839	753	1,592
Villa Obregon	315	473	517	564	1,220	1,506	1,583	3,089
Totals	15,419	19,094	19,092	19,315	29,312	51,597	50,672	102,269

population to be vaccinated, because it was almost all a matter of a closed community

In contrast, the work done by the health offices took place in an open community and with little prior publicity

Table 3 shows the absolute figures of administrations of vaccine Type 3 given by each Social Security clinic, and Table 4 from the same source, shows the relation between vaccinations with Type 1 and Type 3

It is satisfactory to state that the high percentages show not only the control facilities, but also the success in winning back backsliders as well as the confidence the public showed in the product

In Table 5 will be found absolute figures ob-

TABLE 2 POLIOMYELITIS VACCINATION WITH SABIN ORAL VACCINE IN MEXICO CITY AND SIX PRECINCTS OF THE FEDERAL DISTRICT—1959

OFFICE	TOTAL POPULATION	POPULATION UNDER 5 YEARS	FIRST DOSE	COEFFICIENT c_c
HEALTH DISTRICT I	603,601	84 050	12 108	14 40
HEALTH DISTRICT II	302 994	42 419	9,358	22 06
HEALTH DISTRICT III	387,326	54 225	14,689	27 08
HEALTH DISTRICT IV	200 207	28 029	2 123	7 57
HEALTH DISTRICT V	117,355	16 430	1 650	10 04
HEALTH DISTRICT VI	206 139	28 859	3,954	13 70
HEALTH DISTRICT VII	303 410	42 481	8 980	21 13
HEALTH DISTRICT VIII	302 436	42 341	4 533	10 70
HEALTH DISTRICT IX	489 635	68 548	11 124	16 22
HEALTH DISTRICT X	213 000	29 820	6 529	21 89
HEALTH DISTRICT XI	298 220	41 750	5 626	13 47
HEALTH DISTRICT XII	267,932	37 751	5 131	13 59
ATZCAPOTZALCO	315,611	44 185	4,289	9 70
IXTAPALAPA	128 723	18 021	3 329	18 47
COYOACAN	117,608	16 465	2 217	12 69
LA MAGDALENA CONTRERAS	56 884	7 963	1,948	24 46
TLALPAN	55 058	7 707	1 592	20 65
VILLA OBREGON	156,535	21 914	3,089	14 09
Totals	4 522,707	632 958	102,269	16 15

TABLE 3 ORAL POLIOMYELITIS VACCINATION—SABIN STRAIN VACCINATION BY CLINICS
SUB-STAGE FROM 6 APRIL TO 14 MAY 1959

Clinic	Age					Sex		Doses			Total Doses Administered
	6 to 11 Months	1 Year to 1-11/12	2 Years to 2-11/12	3 Years to 3-11/12	4 Years to 4-11/12	Masc	Fem	First	Second	Third	
1	370	455	495	477	1,083	1,440	1,440		2,880		2,880
2	871	1,287	1,258	1,213	1,921	3,377	3,173		6,550		6,550
3	893	1,096	1,099	1,159	2,042	3,144	3,145		6,289		6,289
4	237	399	370	392	564	984	978		1,962		1,962
5	484	806	705	717	1,001	1,837	1,876		3,713		3,713
6	657	987	933	888	1,351	2,456	2,360		4,816		4,816
7	106	143	172	206	360	528	459		987		987
8	315	455	501	533	1,200	1,453	1,551		3,004		3,004
9	404	691	578	686	1,049	1,691	1,717		3,408		3,408
10	246	420	405	408	423	977	925		1,902		1,902
11	816	1,271	1,180	1,078	1,202	2,805	2,712		5,547		5,517
12	223	369	359	353	587	932	959		1,891		1,891
13	190	331	352	347	436	837	819		1,656		1,656
14	259	401	408	418	474	988	1,005		1,993		1,993

TABLE 4 ORAL POLIOMYELITIS VACCINATION—SABIN STRAIN

Second Doses Given by Clinic as Compared with First Doses

Sub-stage from 6 April to 14 May 1959

CLINIC	FIRST DOSES GIVEN	SECOND DOSES GIVEN	
		NUMBER	PERCENTAGE
1	3,098	2 880	92 9
2	6 858	6 550	95 5
3	6,814	6 289	92 3
4	2 067	1 962	94 9
5	3,865	3 713	96 1
6	5,335	4,816	90 2
7	1 129	987	87 4
8	3 089	3 004	97 3
9	3 872	3 408	88 0
10	1,983	1 902	95 9
11	6 270	5 547	88 9
12	1,918	1 891	98 6
13	1,726	1 656	95 9
14	2,000	1,993	99 5
15	1,287	1,193	92 6
30	1,295	1 252	96 7
31	652	619	94 9
Total	53,258	49,662	93 3

tained when the second dose (Type 3) was given by the dependencies of the Ministry of Health and Welfare, and in Table 6, a comparison of the percentages of second doses with respect to the first.

The indices of continuing treatments are lower than in Social Security, *both individually* and collectively The reasons are pending tabulation—which is considered very important because it will serve in the preparation of future work—but it can be expected that the following will have weight

1 Permanent removal from the locality
2. Temporary removal from the city
3 Change of domicile from the health district but within the city
4. Change of domicile in the health district.
5 False domicile.
6 Intermittent illness.
7 Death
8 Refusal

The first two reasons are very important in the case of large cities with a large floating population especially if the length of time between doses is taken into account.

The third and fourth reasons are very important just now because of the changes being made by the municipal authorities (Department of D F) through major urbanization projects, *which affect practically the entire Federal District*. since excepting the precincts of Milpa Alta, Xochimilco, Tláhuac, Cuajimalpa, and Magdalena Contreras, all the rest are actually part of the capital city

The false statement as to domicile is considered more frequent than any other The decision to limit the application to the residents of a health district—essential for checking purposes—*was the original reason for this falsehood* by persons impressed but not convinced Moreover, it is impossible for the public health personnel, without a prior visit to check the domicile, to guarantee that an applicant is part of the population in the district under their jurisdiction

The intermittent illness that contraindicates or interrupts the continuation of the treatment is another cause that cannot be ignored and is more important when there are increases in endemic diseases, epidemic outbreaks, or epidemics.

Death, which may come from one of many causes, should of course be considered

Finally, refusal to continue the treatment may be due to a natural lack of confidence in the unknown, especially when various popular opinions or interpretations enter into the picture

In the figures for Clinic 5, it will be seen that

Table 5 Report on Applications of Sabin Poliomyelitis Vaccine
Dose Second—Date from 6 April to 15 May 1959

	6 to 11 M		1 Year		2 Years		3 Years		4 Years		Subtotal		Total
	M	F	M	F	M	F	M	F	M	F	M	F	
HEALTH DISTRICT I	220	212	224	207	245	241	240	236	418	406	1,347	1,302	2,649
HEALTH DISTRICT II	320	268	329	302	299	340	350	316	359	367	1,663	1,593	3,256
HEALTH DISTRICT III	703	714	637	654	712	738	805	781	1,060	978	3,917	3,862	7,779
HEALTH DISTRICT IV	179	151	197	169	174	168	149	162	188	193	887	843	1,730
HEALTH DISTRICT V	115	106	151	153	119	149	119	120	228	205	762	733	1,495
HEALTH DISTRICT VI	124	130	145	147	200	169	175	143	212	215	856	804	1,660
HEALTH DISTRICT VII	118	122	174	146	183	149	171	136	239	233	918	787	1,705
HEALTH DISTRICT VIII	100	78	105	101	112	125	132	119	172	148	621	571	1,192
HEALTH DISTRICT IX	549	535	571	542	564	514	624	608	1,068	1 011	3,375	3,213	6,588
HEALTH DISTRICT X	232	217	251	266	231	259	234	222	270	284	1,218	1,248	2,466
HEALTH DISTRICT XI	275	275	293	318	299	315	320	331	482	473	1,669	1,712	3,381
HEALTH DISTRICT XII	232	194	250	252	260	266	308	258	301	301	1,357	1,271	2,628
ATZCAPOTZALCO	169	172	184	174	188	208	216	205	360	390	1,123	1,149	2,272
IXTAPALAPA	152	145	139	137	133	117	118	130	281	302	823	810	1,663

Table 5 Report on Applications of Sabin Poliomyelitis Vaccine
Dose Second—Date from 6 April to 15 May 1959
(Continued)

	6 to 11 M		1 Year		2 Years		3 Years		4 Years		Subtotal		Total
	M	F	M	F	M	F	M	F	M	F	M	F	
COYOACAN	83	113	104	113	123	108	103	95	253	207	666	636	1 302
LA MAGDALENA CONTRERAS	54	59	42	40	56	33	57	57	96	80	305	269	574
TLALPAN	19	13	34	38	40	41	44	33	71	61	208	186	394
CLINIC 2	33	30	43	46	43	41	44	33	53	56	216	206	422
CLINIC 3	26	25	22	29	34	48	30	33	28	36	140	171	311
CLINIC 4			5	7	3	3	3	3	4	6	15	19	34
CLINIC 5	7	10	35	19	32	31	38	30	35	12	147	132	279
CLINIC 6	8	8	10	10	10	13	9	10	14	18	51	59	110
Totals	3,754	3,577	3,945	3,867	4 065	4,076	4,322	4,071	6,198	6,015	22,284	21,606	43,890

Table 6 Relationship Between the 1st and 2nd Doses of Sabin Oral Poliomyelitis Vaccine in Mexico City and Five Precincts of D F.—1959

Office	Doses		%
	1st	2nd	
HEALTH DISTRICT I	2,901	2,649	91 219
HEALTH DISTRICT II	3,542	3,256	91 925
HEALTH DISTRICT III	8,419	7 779	92 398
HEALTH DISTRICT IV	1,848	1 730	93 614
HEALTH DISTRICT V	1 650	1 495	90 606
HEALTH DISTRICT VI	1 887	1,660	87 970
HEALTH DISTRICT VII	1 947	1,705	87 570
HEALTH DISTRICT VIII	1 435	1,192	83 066
HEALTH DISTRICT IX	7,259	6 588	90 756
HEALTH DISTRICT X	2 657	2,466	92 811
HEALTH DISTRICT XI	3 708	3 381	91 181
HEALTH DISTRICT XII	3,148	2,628	83 481
ATZCAPOTZALCO	2,563	2,272	88 646
IXTAPALAPA	2,042	1 663	81 439
COYOACAN	1 565	1,302	83 194
LA MAGDALENA CONTRERAS	653	574	87 901
TLALPAN	463	394	85 097
CLINIC 2	481	422	87 733
CLINIC 3	390	311	79 744
CLINIC 4	49	34	69 387
CLINIC 5	275	279	101 454
CLINIC 6	126	110	87 301
TOTALS	49,011	43,890	89 551

TABLE 7 POLIOMYELITIS VACCINATION WITH SABIN ORAL VACCINE IN RELATION TO PERSONS CONTROLLED AFTER THE ADMINISTRATION OF THE FIRST DOSE, MEXICO, D. F.—1959

OFFICE	PERSONS VACCINATED—1ST DOSE	CONTROLLED	%
HEALTH DISTRICT I	2,904	1,826	62 87
HEALTH DISTRICT II	3,542	1,202	33 93
HEALTH DISTRICT III	8 419	990	11 75
HEALTH DISTRICT IV	1,848	1,356	73 37
HEALTH DISTRICT V	1,650	1,170	70 90
HEALTH DISTRICT VI	1,887	1,859	98 51
HEALTH DISTRICT VII	1,947	858	44 06
HEALTH DISTRICT VIII	1,435	763	53 17
HEALTH DISTRICT IX	7,259	1,188	16 36
HEALTH DISTRICT X	2,657	619	23 29
HEALTH DISTRICT XI	3,708	1,012	27 29
HEALTH DISTRICT XII	3,148	926	29 41
ATZCAPOTZALCO	2 563	326	12 71
IXTAPALAPA	2,042	535	16 19
COYOACAN	1,565	480	30 67
LA MAGDALENA CONTRERAS	653	551	84 37
TLALPAN	463	259	55 93
TOTALS	47,690	15,920	33 38

the number of second doses was larger than that for first doses Upon checking, it was learned that persons who had received the first dose elsewhere had been received without requesting of them the pertinent documents The necessary adjustments will be made in the final report

It has already been said that the medical clinics begin the prophylactic work and lack health nursing services, which explains the high number of desertions

Ixtapalapa and Coyoacán are areas with a high proportion of rural dwellers with a slight general and health education, which may explain the percentages there

Up to 13 June the Social Security clinics had administered the third dose (Type 2) to 36,794 children, and up to the 16th of that month the

health centers had done so to 29,971, that is, between the two there was a total of 66,765 treatments completed. If this figure is compared with the total number of second doses (Type 3) 93,552, the percentage is 71.36, which leads to the supposition that in the period remaining until the conclusion of the program—which might be prolonged if necessary but will unquestionably be brief—a very satisfactory figure of complete administrations will be obtained.

Fifty five nurses were used to check on the persons vaccinated. All of these nurses had performed similar work in the Salk vaccination program. With some exceptions they were selected from among the staff of the health districts and assigned to the area with which they were familiar. The epidemiologists and the pediatricians responded to the consultations requested of them.

Table 7 gives the indices of persons controlled after the administration of the first dose at each health center. In some centers almost all persons vaccinated were visited, but the number of further visits was lower. In others, the opposite was true, the number of people visited was smaller, but the number of visits to them increased. The visits were made preferably in the first two weeks after the dose was taken. In any form, the sampling of 33 per cent seems sufficient. The symptomatic manifestations discovered in those vaccinated could not be connected with the administration of the vaccine, but a connection was seen with other intermittent causes. In general, it seems not to have caused local or general reactions.

Final considerations

The conclusions on the work done in Mexico with Sabin oral vaccine can be reached only after the various aspects of the campaign have been compiled and put in order.

For the present, only the following facts can be stated:

1. Oral poliomyelitis vaccine as a campaign procedure is preferred by the public over other methods.
2. It can be administered under the public health programs without any considerable rise in the cost of normal works.
3. It is easy to handle and requires no special equipment.
4. It can be rapidly applied in work done on a large scale.
5. It makes it possible to guarantee the conclusion of a high percentage of the treatments begun, if there is timely and adequate publicity.
6. The percentage of desertions may be lowered by reducing the time between doses or even, if possible, by reducing the number of doses.

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HEALTH DISTRICT X	2,657	619	23 29
HEALTH DISTRICT XI	3,708	1,012	27 29
HEALTH DISTRICT XII	3 148	926	29 41
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IXTAPALAPA	2,042	535	16 19
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DISCUSSION

CHAIRMAN ARMSTRONG The paper presented by Dr Guevara Rojas is now open for discussion

DR SABIN I should only like to remark for the record that what Dr Guevara Rojas has just read to us shows what a tremendous job it was to accomplish what was done there, and all the other places where this work was undertaken, and how much effort has gone into this task

I believe that when we just look at a few tables, we realize how much work and how many people had to sweat and work hard in order to provide the data on those tables, and we can recognize the indebtedness that everyone has to the people who have done all this work

DR BARR· I was pleased to hear mention of the Salk vaccine, because I think that we should be very careful not to go away from this meeting leaving an impression that the use of Salk vaccine is being abandoned in the areas where it has been carried out extensively, particularly in this and other countries We in Minnesota have been pushing its use as hard as we can ever since it became available, and we have a high per cent of our people immunized

We would not want to leave an impression with the public that we are saying now that we have oral vaccine in the experimental stage We could let down and stop using Salk vaccine and have a lapse in what protection we now have

CHAIRMAN ARMSTRONG That certainly is true and especially true since the Public Health Service has not yet approved the living virus

DR. BARR· Even if they did, I think that we should hold on We cannot abandon one tool for another one until the second tool proves to be better in every way and under all conditions

CHAIRMAN ARMSTRONG· That is right Dr Lebrun

DR LEBRUN I should like to emphasize what Dr. Barr has just stated, and to add that Salk vaccine has different indications from live vaccine, indications that very often are conditioned by economic and psychologic factors There is also the fact that Salk vaccine requires a fairly long time to ensure a population's protection and, as I have stated, there is the psychologic aspect related to injections, which are not always accepted by primitive populations Moreover, in the case of mass vaccinations in primitive populations, carried out with a more or less trained medical staff, there arises, with the injection itself, the danger of transmitting other viruses as well It has been well established that Salk vaccine has rendered and will continue to render an enormous service but it is not applicable under certain special conditions particularly in the economically less developed countries I just wished to stress this fact

CHAIRMAN ARMSTRONG· Dr Sabin

DR SABIN May I be permitted one comment that has to do with one practical point that Dr Guevara Rojas phrased Dr Guevara Rojas said that in the procedure involved in taking oral temperatures, the thermometers were kept in 95 per cent alcohol

Now, 95 per cent alcohol does not destroy poliovirus

What I have been thinking is this On the basis of the small samples that we have, we could very conservatively say that perhaps 10 per cent of the children at that time in Mexico City, who were going to the health clinics to get oral vaccine, were probably carrying poliovirus

Now that, I know is predominantly in the rectal swabs, but we also studied pharyngeal swabs, and while all the pre vaccine pharyngeal swabs had no virus in them, we did have one child that was apparently in the first stages of infection with Type 1, at a time that it came to get Type 2 We followed along with the pharyngeal swabs in this child and were able to show that for a period of the first six days the Type 1 virus was demonstrable only in the rectal swabs Beginning with the sixth day the

virus was also demonstrable in the pharyngeal swabs for a subsequent period of days.

I know from previous studies that when virus is demonstrable in the pharynx there is not very much in the mouth.

I do not want to leave the impression that this constitutes a means of transmitting the wild viruses, but, at the same time, I think that, if oral thermometers are going to be used, some system ought to be devised to put those thermometers in solutions that can quickly inactivate poliovirus, and some other viruses

A solution that I can recommend from personal trials—although I am sure there must be others equally good or maybe better—is hydrogen peroxide. I know that hydrogen peroxide, in tests that I carried out many years ago, came within one minute of inactivating poliovirus. I would therefore suggest that, when such things are done in the future, alcohol not be relied upon for using thermometers over and over again, but that use be made of some strong oxidizing agent—hydrogen peroxide or potassium permanganate—can be used

I am not suggesting this was an important source of transmitting infection, but I would like to suggest that it be kept in another solution in the future

Dr. Soper Mr Chairman, we have heard this week a series of reports of work done only after official action by the responsible government authorities approving the use of the oral vaccine

In Minnesota the work was approved by the State Board of Health and, at a critical point, by the Governor of the State.

The work in Colombia was done only after the Ministry of Health, the Director of Health of Colombia, and the Secretary of Health of the Province of Medellin had officially approved the use of live poliovirus vaccine as a public health measure

Among the reports presented here is that of the Minister of Health of Costa Rica, who took the responsibility, as the chief health officer of his country, in authorizing the use of live virus vaccine

The report from Managua, Nicaragua, was likewise the report of the administrative health officers of the country

We know that the work in the Belgian Congo was from the beginning undertaken as an official program of the Belgian Government

We have heard from the USSR representatives that the expanded program there has been approved by the Ministry of Health

I shall not cite the other instances, but I do want to point out that most of the reports presented here are not based only on the discussion between individuals who have had laboratory experience with the virus and have then convinced other laboratory workers in other countries that this is something which merits further trial.

We have passed far beyond that stage, and have heard this week a number of reports on official programs, one of which at least, the program in Costa Rica, was begun with the deliberate purpose of making it a continuing nation-wide program for the immunization of all the children of the country with live poliovirus vaccine

18. MASS VACCINATION PROGRAM WITH LIVE ATTENUATED POLIOMYELITIS VIRUS IN MONTEVIDEO, URUGUAY*

DR. JUAN JOSÉ LEUNDA, DR. ENRIQUE M. CLAVEAUX, DR. ALBERTO BERTOLINI, DR. GABRIEL GONZÁLEZ DANRÉE, DR. VICENTE SÁENZ BRIONES, DR. JUAN C. BACIGALUPI, DR FEDERICO J. SALVERAGLIO, DR. RICARDO CARITAT, DR JOSÉ ARTURO LORENZO, DR. JUAN A. BORELLI, MR. HÉCTOR C. TOSI, DR. ADOLFO MORALES, DR. JOSÉ SARALEGUI, AND DR. OSVALDO LUZARDO

DR LEUNDA *(presenting the paper)* Uruguay, a nation of southeast South America, is located between the 30th and 35th parallels of latitude south and the 53rd and 58th of longitude west It borders on two large countries to the west the *Argentine Republic*, from which it is separated by the Uruguay River, to the north and east, the United States of Brazil The fact that the Uruguay-Brazil frontiers are, for the most part, mere dividing lines makes for a constant flow of inhabitants from one country to the other Uruguay's climate is generally temperate, there being no extreme temperatures, but there are sudden changes in the weather and frequent winds The racial stock is Caucasian Socio economic conditions are favorable The general epidemiology is very good the country being free from yellow fever, cholera, bubonic plague malaria, smallpox hookworm disease, and typhus Its normal epidemiology consists of eruptive diseases common to childhood, grippe, and other affections of no great importance Tuberculosis and syphilis are clearly on the decline

Population Data

The country's total population is estimated at 2,614,755 Of that total, 950 000 live in Montevideo, the capital of the Republic The distribution of the population in Montevideo and in the country, by age, up to 15 years and over, is shown in Table 1

Poliomyelitis in Uruguay

Identification of poliomyelitis in its epidemic form dates back only to 1906 in Uruguay. From that date to the present there have been a number of epidemic outbreaks of varying seriousness, the 1954 55 epidemic, with 524 cases, having the highest morbidity rate, and the most recent outbreak, with 162 cases, being the one recorded in 1958 59 An important outbreak, with 284 cases, occurred in 1936 37

TABLE 1 POPULATION OF URUGUAY, BY AGE ESTIMATES FOR 1955

AGES	ENTIRE COUNTRY	DEPARTMENT OF MONTEVIDEO
Total	2 614 775	950 000
Under 1 year	59 878	21 850
1 to 4 years	251 541	91,200
5 to 9 years	288 671	105 450
10 to 14 years	282,657	102 600
15 years and over	882,747	321 747

From 1906 to the present, the number of poliomyelitis cases has averaged 100 annually, with no appreciable difference in the figures for the capital and those for the rest of the country All figures given refer to paralytic cases Figure 1 traces the various epidemic waves that occurred in the aforementioned period

Incidence according to geographical area and distribution, by month, during the past ten years

The increased accuracy in relating cases to the calendar months in which they occurred in the past ten years is reflected in figures 2, 3, and 4

* Report of the National Committee against Poliomyelitis, Ministry of Public Health, Montevideo, Uruguay.

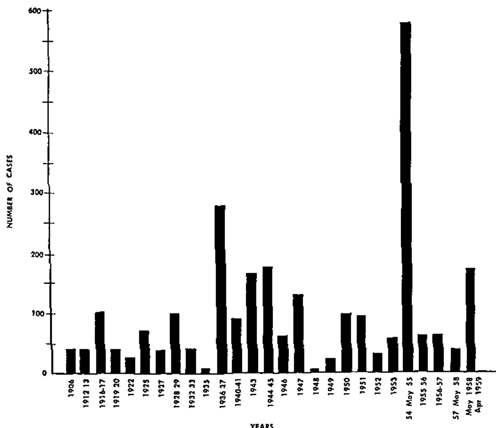


FIG 1 Cases of Paralytic Poliomyelitis, 1906 1959

Those figures indicate that the disease takes the form of epidemic outbreaks at a time generally coinciding with the period of high temperature from November to March

Rates per 100,000 inhabitants

The poliomyelitis rates per 100,000 inhabitants vary between 1 08 and 3 88 A clear exception was the rate of approximately 20 per 100 000 inhabitants, which was reached during the 1954-55 epidemic outbreak Table 2 illustrates this point

Incidence by age

Judging by our clinical epidemiology, the incidence of poliomyelitis in our environment is highest, in terms of percentages, among those under 15 years of age (85 5 per cent) Within the 0-15 year-group the age most affected is the 0 3-year-group (44 4 per cent)

It is interesting to note that, in Uruguay, the incidence of poliomyelitis in adults over 30 years of age is barely 1 per cent (See Tables 3 and 4)

Orally administered vaccine

Administration of vaccine by mouth was begun in Uruguay in May 1958 by agencies of the Departmental Council of Montevideo It was continued during the months of June, July, August, and September During that period 18,000 persons were fed

In September 1958 the National Government authorized the controlled application of the vac

18. MASS VACCINATION PROGRAM WITH LIVE ATTENUATED POLIOMYELITIS VIRUS IN MONTEVIDEO, URUGUAY*

Dr. Juan José Leunda, Dr. Enrique M. Claveaux, Dr. Alberto Bertolini, Dr. Gabriel González Danrée, Dr. Vicente Sáenz Briones, Dr. Juan C. Bacigalupi, Dr. Federico J. Salveraglio, Dr. Ricardo Caritat, Dr. José Arturo Lorenzo, Dr. Juan A. Borelli, Mr. Héctor C. Tosi, Dr. Adolfo Morales, Dr. José Saralegui, and Dr. Osvaldo Luzardo

Dr. Leunda (*presenting the paper*): Uruguay, a nation of southeast South America, is located between the 30th and 35th parallels of latitude south and the 53rd and 58th of longitude west. It borders on two large countries: to the west the Argentine Republic, from which it is separated by the Uruguay River, to the north and east, the United States of Brazil. The fact that the Uruguay-Brazil frontiers are, for the most part, mere dividing lines makes for a constant flow of inhabitants from one country to the other. Uruguay's climate is generally temperate, there being no extreme temperatures, but there are sudden changes in the weather and frequent winds. The racial stock is Caucasian. Socio economic conditions are favorable. The general epidemiology is very good, the country being free from yellow fever, cholera, bubonic plague, malaria, smallpox, hookworm disease, and typhus. Its normal epidemiology consists of eruptive diseases common to childhood, grippe, and other affections of no great importance. Tuberculosis and syphilis are clearly on the decline.

Population Data

The country's total population is estimated at 2,614,755. Of that total, 950,000 live in Montevideo, the capital of the Republic. The distribution of the population in Montevideo and in the country, by age, up to 15 years and over, is shown in Table 1.

Poliomyelitis in Uruguay

Identification of poliomyelitis in its epidemic form dates back only to 1906 in Uruguay. From that date to the present there have been a number of epidemic outbreaks of varying seriousness, the 1954-55 epidemic, with 524 cases, having the highest morbidity rate, and the most recent outbreak, with 162 cases, being the one recorded in 1958-59. An important outbreak, with 284 cases, occurred in 1936-37.

Table 1. Population of Uruguay, by Age. Estimates for 1955

Ages	Entire Country	Department of Montevideo
Total	2 614,775	950 000
Under 1 year	59,878	21 850
1 to 4 years	251 541	91,200
5 to 9 years	288,671	105 450
10 to 14 years	282,057	102 600
15 years and over	882 747	321 747

From 1906 to the present, the number of poliomyelitis cases has averaged 100 annually, with no appreciable difference in the figures for the capital and those for the rest of the country. All figures given refer to paralytic cases. Figure 1 traces the various epidemic waves that occurred in the aforementioned period.

Incidence according to geographical area and distribution, by month, during the past ten years

The increased accuracy in relating cases to the calendar months in which they occurred in the past ten years is reflected in figures 2, 3, and 4.

* Report of the National Committee against Poliomyelitis, Ministry of Public Health, Montevideo, Uruguay.

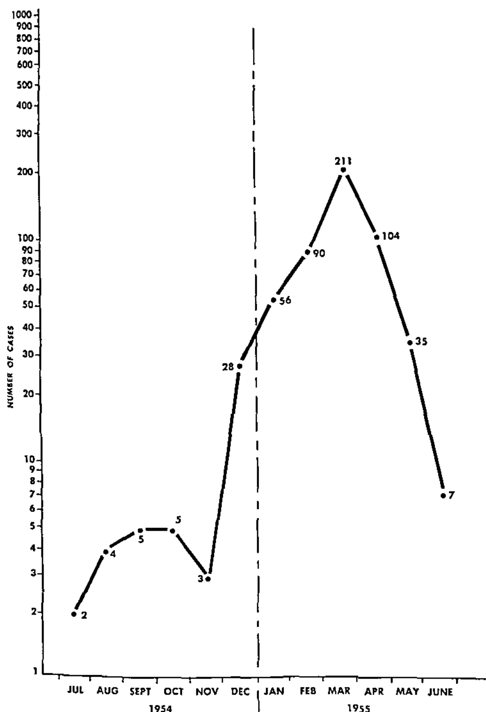


FIG 3 Poliomyelitis cases July 1954 June 1955 524 cases Semilogarithmic Scale

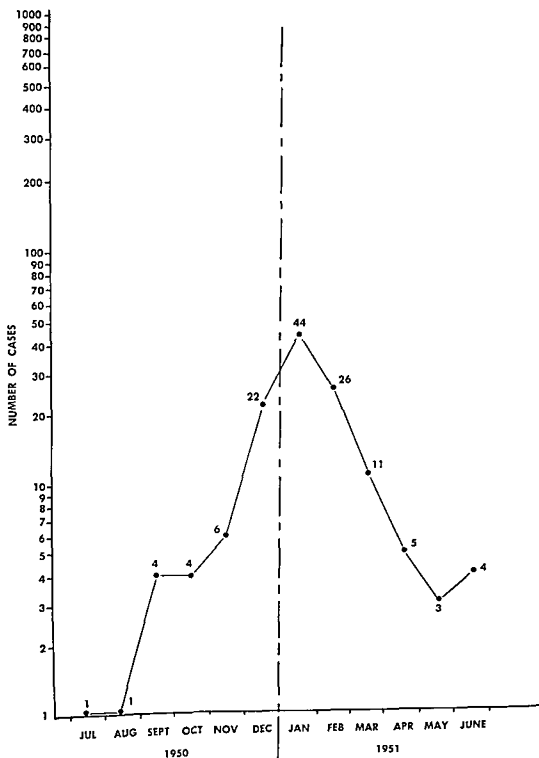


FIG 2 Poliomyelitis cases July 1950 June 1951 Semilogarithmic Scale

TABLE 2 POLIOMYELITIS CASES AND RATES PER 100,000 INHABITANTS FOR THE ENTIRE COUNTRY AND FOR THE DEPARTMENT OF MONTEVIDEO—1950 TO 1958

YEAR	ENTIRE COUNTRY			DEPARTMENT OF MONTEVIDEO		
	ESTIMATED POPULATION	CASES	RATES	ESTIMATED POPULATION	CASES	RATES
1950	2,406,894	79	3 28	884,893	34	3 84
1951	2,449,081	95	3 88	897,547	36	4 01
1952	2,490,000	27	1 08	900,382	9	0 99
1953	2,535,319	56	2 21	923,402	30	3 25
1954	2,579,165	85	3 30	936,607	25	2 67
1955	2,611,775	528	20 19	950 000	264	27 79
1956	2,658,442	71	2 67	963,585	18	1 87
1957	2,702,838	49	1 81	977,361	22	2 25
1958	2,747,975	162	5 90	991,340	87	8 78

TABLE 3 CASES OF PARALYTIC POLIOMYELITIS REPORTED, CLASSIFIED FROM THE EPIDEMIC OUTBREAK OF 1958-1959

AGE	TOTAL	DEPARTMENT OF MONTEVIDEO	ALL OTHER DEPARTMENTS
Total	162	87	75
Under 3 years	96	52	44
3– 5 years	20	15	5
6–14 years	20	7	13
15–19 years	9	5	4
20 years and over	6	3	3

TABLE 4 DISTRIBUTION OF POLIOMYELITIS CASES, BY AGE—EPIDEMIC OF DECEMBER 1954-MAY 1955

AGE	NUMBER OF CASES	PERCENTAGE OF TOTAL NUMBER
Total	524	100 0
Under 1 year	53	10 1
1– 2 years	180	34 4
3– 5 years	104	19 8
6–13 years	112	21 4
14–19 years	38	7 3
20–29 years	30	5 7
30 years and over	7	1 3

cine From then on, the activity was carried out under the direction of the National Committee Against Poliomyelitis, the authors of this report The Committee sought to act in accordance with a program which could be put into effect only partially, owing to circumstances that will be explained below

From the beginning of the oral vaccination program to the present, 218 624 persons were fed three doses of the vaccine, 26,422 have had only two doses, and 29,676, only one dose Table 5 shows the distribution of the vaccine applications, by age groups.

Information on some 40,000 persons given the vaccine by private physicians is not included in the table, inasmuch as the pertinent data have not yet been furnished by those physicians.

From the outset of the program up to the present, the vaccine used was furnished by Lederle Laboratories, in the usual capsule form, the virus types being distinguished by different colors In accordance with recommended procedure, the vaccine was stored in deepfreezes at temperatures below −20°C Each day the

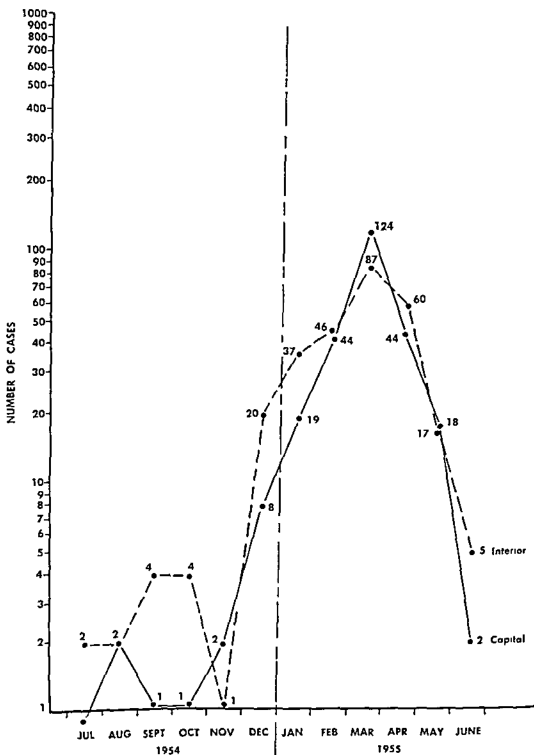


FIG. 4 Poliomyelitis cases July 1954 June 1955 Semilogarithmic Scale

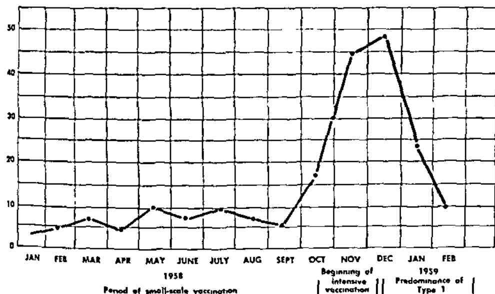


Fig. 5. Paralytic Poliomyelitis Cases 1958

TABLE 6. MONTEVIDEO PRE-EPIDEMIC SEROLOGIC SURVEY IN MISCELLANEOUS GROUPS FROM VARIOUS DISTRICTS IN 1958 (97 PERSONS)

Age Distribution, in Per Cent

Age Groups						
1–4	5–9	10–14	15–19	20–24	25+	?
4%	26%	43%	8%	1%	3%	15%

Distribution of Antibody Titers in Percent

Virus Type	<4	4	8	16	64	256	>1024
1	27.8	3.2	4.1	14.5	31.1	12.4	7.2
2	18.6	4.1	13.4	12.4	18.6	23.7	9.3
3	36.1	8.2	6.2	22.7	18.6	5.2	2.1

Distribution of Sero-Negatives

Virus Type	Number
1	8
2	3
3	17
1, 2	5
1, 3	9
2, 3	4
1, 2, 3	5
Total Negatives by Type	
1	27
2	17
3	35
	Total 79

TABLE 5 NUMBER OF PERSONS WHO RECEIVED POLIOMYELITIS VACCINE BY MOUTH

AGE	DOSES		
	ONLY THE FIRST	FIRST AND SECOND	FIRST, SECOND, AND THIRD
Total	29,676	26,422	218,624
Under 3 years	5,340	2,322	25,054
3– 5 years	3 902	4 085	26 290
6–15 years	7,943	9,916	93,198
16–19 years	5,904	7,432	34,891
20 and over	6 587	2 667	38,514
Pregnant women	—	—	647

amounts needed for immediate use were withdrawn from the stock and delivered to the vaccination centers located within the limits of the Department of Montevideo.

Those centers were equipped with regular refrigerators, which kept the vaccine at the proper temperature until it was used A periodic check was made of the potency of the vaccine

Pathological manifestations observed among vaccinees

1 *Nonparalytic type*

(a) *Digestive disorders* Diarrhea, vomiting, loss of appetite were observed

(b) *Skin disorders* Erythemas of various types but of little significance

(c) *General* Fever, weakness myalgia, dizziness, headache, grippe-like syndromes, etc.

Those symptoms had no clinical importance and occurred infrequently In a group of 20-000 carefully observed school children, the incidence of the above-mentioned disturbances was on the order of 2 5 per 1 000

2. *Paralytic type*

In the course of the vaccination, seven cases of paralytic syndromes diagnosed as poliomyelitis were observed. Of those seven cases, five were patients who had been vaccinated with Type 2 virus and whose stool specimens showed the presence of Type 1 virus The sixth case had been fed Types 1 and 3 and his stool specimen likewise indicated the presence of Type 1. The seventh and final case had been vaccinated with Type 1 virus, which was also recovered from his stool specimen All the clinical and laboratory data on the foregoing cases will be presented later in this Conference by Dr Roca-García

The 1958-59 outbreak in relation to the vaccination

Four months after the oral vaccination program was begun in the Department of Montevideo, an epidemic occurred which affected equivalent numbers of inhabitants of that department (where the oral vaccination was being carried out) and of the other departments (where the vaccine was not being administered) The outbreak started in the season during which such epidemics usually occur in Uruguay It declined quite sharply four months after it had begun, during a hot season, when its prolongation would seem likely

The characteristics of that outbreak are shown in Figure 5

Biological tests

The biological tests were based, first, on the isolation and classification of the viruses Of the 105 strains isolated, 68, or 64 7 per cent, were of Type 1, 34, or 32 2 per cent were identified as Type 2, and 3, or 2 8 per cent, belonged to Type 3 Thus, in this epidemic there was an apparent predominance of Type 1 virus The data we have collected in connection with virological tests concern cases that developed in connection with the epidemic or in persons who were vaccinated during the epidemic

Prior to the vaccination, 375 blood specimens were taken Of them, 83 were paired Moreover, 128 specimens were drawn from vaccinated persons from whom no pre-vaccination specimens were obtained The result of the pre-vaccination serologic survey based on 97 samples is shown in Table 6

DISCUSSION

CHAIRMAN ARMSTRONG: The Secretariat has an announcement

DR MARTINS DA SILVA: I think you have all received the sheets in which we ask your co-operation in sending in, as soon as possible, the corrections to your papers. The Pan American Sanitary Bureau intends to publish in English the Proceedings of the Conference on Live Poliovirus Vaccines as soon as possible after the close of the Conference.

The participants should fill out the question naire included and return it to us before the Conference adjourns We request also the discussants to do likewise The material presented in slides and not included in the documents should be turned over to us for inclusion in the Proceedings

CHAIRMAN ARMSTRONG: We will now proceed with the discussion of Dr Leunda's paper Dr *Soper*

DR SOPER Mr. Chairman, I shall not discuss Dr Leunda's paper, but would like to make a slight correction with regard to the participation of the Pan American Sanitary Bureau in the program in Uruguay.

The initial drive for using a live virus polio vaccine in Uruguay came from Dr Enrique Claveaux, ex-Minister of Health of Uruguay, *who in February of 1957* was the representative of Uruguay at the Regional Health Meeting of the River Plate Countries (Argentina Brazil, Paraguay, and Uruguay)

The representative of Uruguay raised the question of what to do about poliomyelitis, and informed the meeting that he was already in contact with Dr. Cox and was anxious to work with the live polioviruses in his country, remembering at that time the sad experience that Uruguay had had with poliomyelitis just two years before, as Dr Leunda has told us this morning

I was able to inform the delegates at that meeting of some of the results of live poliovirus vaccine work up to that time, having been just a few weeks before rather thoroughly indoctrinated by Dr Albert Sabin, with the able *assistance of Dr John Paul*

Early in 1958 I visited Uruguay and some other Latin American countries, offering the collaboration of the Pan American Sanitary Bureau in vaccination with live poliovirus vaccine and offering each country a choice of strains to be used, those distributed by Dr Cox, or the strains developed by Dr Sabin.

At the time of, and for some months following, my visit in February of 1958, the Minister of Health of Uruguay did not approve the use of live poliovirus vaccine in the country. The Uruguayan program was really developed by the municipal authorities of the city of Montevideo After a certain time the Ministry did take it over and made it a national program.

But the Pan American Health Organization has not participated technically in the program in Uruguay, nor has it had the same intimate relationship that has existed *with the programs* in Minnesota, in Colombia, in Nicaragua, and in Costa Rica

Mr Chairman, the credit for the initial drive for vaccination in Uruguay should go to Dr. Claveaux, who has been very much concerned with this problem for a number of years

Conditions under which the oral vaccination with live attenuated virus was administered

We have already reported that the oral vaccination with attenuated virus was initiated by agencies of the Departmental Council of Montevideo in May of last year and continued by that body until September of the same year. During that period the National Committee Against Poliomyelitis was unable to go into action for lack of authorization by the National Executive Branch Such authorization was granted on 16 September 1958

Prior to the Executive Decree, the vaccination was administered with the collaboration of private physicians, who reported on the tolerance of the vaccine used

From the date on which the Executive Branch issued the Decree, the National Committee Against Poliomyelitis assumed supervision of the oral administration of the poliomyelitis vaccine, with the intention of carrying out the program drawn up on 10 July 1958

As the foregoing indicates, the National Committee did not tackle the problem from the very beginning but had to take into account the existence of 18,000 persons who had recently been vaccinated and who showed no signs of illness attributable to the vaccine There was, therefore, no reason for interrupting the vaccination program

It was under those conditions that the National Committee set out to continue the vaccination program already begun, applying the plan of controlled immunization which it had drawn up

Several circumstances arose at that time to change the course of the vaccination campaign (1) the epidemic outbreak which occurred in October; (2) the alarm which spread through the population as a result of the notoriety which followed the death of a well-known athlete, (3) the insufficient supply of Salk vaccine to meet the public's demand, and (4) the ample supply of oral vaccine furnished by Lederle Laboratories

This series of circumstances serves to explain the reason for our carrying out a mass vaccination program in Montevideo without biological data proportional to such a broad-scale campaign

Final conclusions

As final conclusions we may state:

1. From the clinical observation of the vaccinees it would appear that, to date, there have been no significant pathological manifestations attributable to the vaccine.

2 As for the antigenic value of the vaccine, we reserve our opinion until we learn the results of the laboratory studies currently under way. We recognize, however, that, even with those results, our contribution in this area of the work will not be as far-reaching as we might have hoped, owing to the reasons given above

3 With regard to the value of the vaccination, as judged by the epidemiological results, we feel that a conclusion on this score would be premature, inasmuch as the vaccination is very recent and it is still under way Yet we believe that in the future the mass vaccination in the city of Montevideo will furnish a pattern for judging the effectiveness of the immunity conferred by the vaccine

We base this belief on the fully documented information we have collected for the past 50 years on the normal poliomyelitis epidemiology of the Department of Montevideo, as the documents included in this report show.

Moreover, the fact that we have limited the oral vaccination to the Department of Montevideo will permit us to compare the epidemiological evolution of infantile paralysis with that in the other departments of the Republic, where attenuated virus vaccine has not been administered and whose epidemiology is likewise a matter of record

serum dilutions against 100 to 500 LD_{50} of virus from either of the two Coxsackie virus isolates

Virus identification and assay: Virus content of stool specimens was assayed by inoculation of 10 or 20 per cent stool suspensions into four to six MK and HeLa-cell tissue culture tubes. When cytopathogenicity was evident, the isolate was tentatively assumed to be poliovirus and neutralization tests for identification were performed in either MK or HeLa cell cultures Before a stool sample was considered negative for cytopathogenic viruses, three or four blind passages were made.

To isolate Coxsackie virus, stool sample suspensions were injected in 0 03 ml amounts into separate litters of suckling mice or hamsters, by each of the following routes, intracerebral (IC), intraperitoneal (IP), and subcutaneous (SC) A stool sample was considered negative for Coxsackie virus only after one or two blind passages had been made. When evidence of virus activity was observed, one or two subsequent passages were made in mice or hamsters using either nervous tissue or a carcass suspension injected SC

Monkeys used for pathogenicity tests of Coxsackie or poliovirus isolates were observed for 21 to 28 days. Any animal found dying or dead during the observation period was autopsied and its spinal cord was taken for pathology studies In some cases, small portions from the cervical and lumbar regions were reserved for virus isolation attempts Surviving animals were sacrificed at the end of the observation period, and their cords and brains also were examined histopathologically. Blood samples were collected from each monkey before its intramuscular inoculation and at the end of the observation period

Monkey cervical and lumbar cord enlargements were examined and scored for pathology according to the criteria suggested by Melnick.[2] The microscopic sections for this study were prepared by a method, shown schematically in Figure 1 and to be described elsewhere by Dr Orsi, which permitted easy and rapid orientation of cord sections, scanning of large segments of the cord, the location of the needle track in monkeys inoculated intraspinally, and evaluation of associated neuronal damage and inflammatory response following inoculation of monkeys by various routes In addition, in 25 of the 54 monkeys studied, numerous sections at all levels of the central nervous system were made. These levels included the cerebral cortex, basal ganglia, thalamus, midbrain, cerebellum, pons and medulla.*

Separate 10-per-cent suspensions from the cervical and lumbar cord regions of inoculated monkeys were assayed for virus content in suckling mice or hamsters and in MK and HeLa-cell cultures, by the procedure described above for stool assay To determine the presence of viremia in monkeys which had been injected with Coxsackie isolates simultaneously by the IC and intramuscular (IM) routes, blood serum specimens obtained two to six days after inoculation were injected into suckling mice or hamsters, by the stool assay procedure

To determine the degree of neurotropism of the Type 3 and Type 1 poliovirus strains recovered from Subjects 10-C and 11-U (GLV and RMS) who had been fed, respectively, Type 3 and Type 1 monovalent oral vaccines, groups of five monkeys were inoculated IC with each isolate. Each animal received 1 0 ml of undiluted fluid, with virus content of 10^7 TCD_{50}, from the second or third MK tissue-culture passage The inocula had previously been proved to be free of Coxsackie virus by their inactivity following IC IP or SC inoculation in suckling mice

The pathogenicity of the Coxsackie virus isolates, after testing for the presence of poliovirus by three consecutive blind passages in MK tissue cultures, was investigated by the inoculation of monkeys, by each of several routes with 10-per cent carcass suspensions from second or third suckling mouse passages Control monkeys were inoculated intraspinally (IS) with a 10-per-cent carcass suspension of normal suckling mice.

The pathogenicity of a dual infection with the Coxsackie A-4 and poliovirus Type 3 strains isolated from Subject 10-C was determined by inoculating one group of monkeys first IC, with 1 0 ml. representing 10^7 $TCID_{50}$ of the third MK tissue-culture passage of Type 3 poliovirus, and five days later IM with 1 0 ml. of 10-per-

* The authors are grateful to Dr George A Jervis, Letchworth Village, Thiells, N Y, for examination of the brains and some of the spinal cords

19. LABORATORY STUDIES ASSOCIATED WITH THE FIELD TRIALS OF ORAL POLIOVIRUS VACCINE

Manuel Roca-García, Ernest V. Orsi, Floyd S. Markham, Juan C. Bacigalupi, Hanna Doany, Héctor C. Tosi, Victor J. Cabasso, Arden W. Moyer, and Herald R. Cox*

Dr. Roca-García *(presenting the paper)* This report describes efforts of the Viral and Rickettsial Research Section of Lederle Laboratories to determine whether the vaccine strains of poliovirus administered in field trials were casually related to the clinical manifestations following oral vaccination that were reported in four subjects in Colombia and nine in Uruguay

Clinical data for a majority of the cases were submitted by local attending physicians In a few instances, information was obtained directly by the medical personnel engaged in the trials For purposes of the study, numbers were assigned to the cases in the order of the date of onset of illness, with "C" designating Colombia cases and "U" those from Uruguay Efforts were made to obtain clinical specimens and histories of all subjects as soon as possible after the onset of illness, but sometimes important details were lacking or the full cooperation of patients or their parents was not given

MATERIALS AND METHODS

Clinical specimens Samples of stool and of blood serum were collected in screw-capped jars and vials as soon as possible after manifestation of clinical signs, and were stored at −20°C and transported with dry ice In some cases specimens also were collected from household contacts of the patient.

Most stool samples were processed by public health laboratories in the countries where the clinical trials were conducted, and 10 or 20 per cent suspensions were sent to Lederle Laboratories, Pearl River, N Y. A few of the isolations of poliovirus strains were done by virologists in the country of origin, and their findings were confirmed in Pearl River

Animals For Coxsackie virus studies, one- to two-day-old Swiss mice and Syrian hamsters, as well as adult mice weighing 10 to 12 grams, were used Pathogenicity tests of Coxsackie and poliovirus isolates were done in cynomolgus monkeys weighing between 4 and 6 pounds.

Tissue cultures Monkey-kidney (MK) and HeLa-cell tissue culture monolayers were used for virus isolation and assay.

Sera Hyperimmune sera for Coxsackie Group A viruses were kindly supplied by Dr Karl Habel of the National Institutes of Health, Washington. D C Hyperimmune sera for each of the three poliovirus types were the standard antipoliovirus sera routinely used in our laboratories

Neutralization tests. For identification of poliovirus in MK or HeLa-cell tissue culture, the cytopathogenicity technique was used Undiluted material from each isolate, or 1.10 dilutions of it, was mixed with undiluted hyperimmune serum for each poliovirus type, or with normal control serum, in equal volumes After incubation for 1 hour at 37° C., each serum-virus mixture was inoculated in 0 1 ml amounts into each of three tubes of MK or HeLa cell cultures, and the tubes were incubated for six days

For poliovirus antibody determinations, tests were performed as described by Cox *et al* [1]

For Coxsackie virus identification, serial 10 fold dilutions prepared from carcass suspensions, representing the second or third suckling mouse passage, were mixed with equal volumes of Coxsackie A type-specific immune serum or with normal mouse serum and, after incubation for one hour at 37°C., were inoculated in *0 03 ml* amounts into each of six to eight suckling mice or hamsters All inoculated animals were observed for 10 days Coxsackie antibody determinations were carried out with two-fold

* Drs Roca-García, Orsi, Markham, Cabasso, [illegible] and Rickettsial Research Sec[illegible]

cent carcass suspension containing 10^6 mouse LD_{50} from the third suckling mouse passage of Coxsackie A-4 virus.

Virus titrations: Titrations for Coxsackie virus present in the clinical specimens were carried out by SC inoculation in suckling mice or hamsters of ten-fold dilutions in 0 03 ml amounts. Titrations of the poliovirus present in clinical specimens were done in MK or HeLa cells. End points were calculated by the method of Reed and Muench.[3]

Observations

In addition to the clinical history summaries of the individuals who showed signs of illness after ingestion of poliovirus vaccine, Table 1 records the instances of virus isolation from their stools and the poliovirus antibody titers of their sera.

Virus isolations: Stool specimens were received from eight of the Uruguayan and three of the Colombian subjects. From six of the Uruguayan and one of the Colombian specimens, Type 1 poliovirus alone was isolated. In addition, Type 1 poliovirus in association with a Coxsackie A virus, Type 4, was isolated from one Uruguayan specimen (Subject 11-U). Another Coxsackie A-4 strain, in association with Type 3 poliovirus, was isolated from one Colombian specimen (Subject 10-c). The other two of the 11 stool specimens, one from Uruguay and one from Colombia yielded no virus.

Characteristics of Coxsackie virus isolates: Inoculated into suckling rodents by the IC, IP or SC route, both Coxsackie virus strains produced the flaccid paralysis typical of Coxsackie Group A viruses as described by Dalldorf and Sickles[4] and by Melnick *et al*.[5] The incubation period varied from two to six days, depending on the amount of virus injected. In general, the animals died 24 hours after the development of paralysis. Greater concentrations of the virus inoculated into mice weighing 10 to 12 grams, by any of the routes mentioned above or intraspinally (IS), produced no clinical manifestations.

Histological studies of tissue from newborn mice paralyzed by these isolates revealed a myositis characterized by hyalin degeneration of the striated muscles. No lesions were observed in the central nervous system (CNS) or in other organs. Carcass or brain and cord suspensions from second or third suckling mouse passages had titers of 10^6 to 10^7 per 0 03 ml when titrated SC in two-day-old mice or hamsters.

Identification and study of Coxsackie isolates: The probable presence of a Coxsackie Group A virus was indicated by the susceptibility of suckling mice and hamsters, inoculated IC, IP or SC, and by the hyalin degeneration demonstrated in their skeletal muscles. Final identification was made by neutralization tests. Tables 2 and 3 present the results of these tests with the two virus isolates used versus immune sera known to be specific for various types of Coxsackie A viruses. It can be seen that both virus isolates were definitely neutralized by the Coxsackie A-4 immune serum.

Table 4 summarizes the investigation in monkeys of the pathogenicity of the Coxsackie virus isolates from Subjects 10-C and 11 U. None of the monkeys inoculated IC, IM or IS showed any definite clinical signs, but three of the four monkeys inoculated both IC and IM showed viremia on the second day after infection only.

Virus was recovered from suspensions of the spinal cords of monkeys No H-723, inoculated IC, and No H-939, injected IM, both of which were found dead on the fifth day after infection. No virus was recovered from spinal cord suspensions of monkeys Nos H 728, sacrificed on the sixth day, H 727, found dead on the tenth day, H 929, found dead on the 11th day, or H-940, found dead on the 15th day. No attempt was made to recover virus from the other monkeys.

The results of the investigation of Coxsackie A-4 and poliovirus antibody response in monkeys inoculated IM with the Coxsackie virus strains isolated from Subjects 10 C and 11 U are presented in Table 5. It can be seen that all of the monkeys developed specific neutralizing antibodies for Coxsackie A-4 virus, and none of the monkeys tested developed poliovirus antibodies.

The results of the histopathological examination of spinal cord sections from monkeys used to test the pathogenicity of the Coxsackie virus isolated from Subject 10 C are summarized in Table 6. It can be seen that this isolate induced inflammatory changes and neuronal degeneration resembling the lesions of poliomyelitis.

Of the six monkeys inoculated IM (see Figs. 2-A and 2 B) only two showed definite perivas

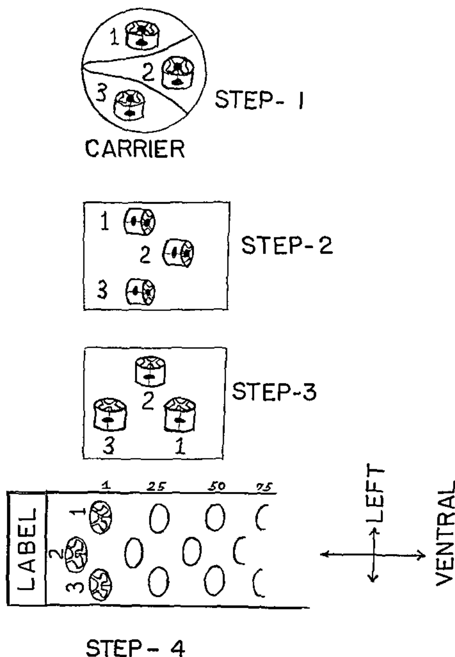


FIG. 1 Method of sectioning cervical and lumbar enlargements of monkeys used in tests

5—U LPB 14 yrs	12/20/58	Quadriplegia	1	—	12/30/58	12/30 5/5/59	1 512 1.16
			2	11/14/58	—	12/30 5/5	1 32 1 1024
			3	12/4/58	—	12/30 5/5	1 512 1 64
8—U JC 4½ yrs	12/27/58	Facial paralysis	1	—	1/4/59	1/4	1 1024
			2	12/15/58	—	1/4	1 1024
			3	—	—	1/4	1 32
13—C MGD 7 mos	3/15/59	Fever, paralysis left leg	1	—	3/21/59	3/21	1 128
			2	9/26/58	—	3/21	1 32
			3	1/26/59	—	3/21	1 512
9—U MCDL 24 yrs	1/4/59	Headache, vomiting, intestinal upset Sore throat, angina pultaceous Paresis, left leg	1	12/29/58		1/20/59	1 512
			2	—		1/20/59	1 8
			3	—	(1)	1/20/59	<1 4
7—U EDTR 25 yrs	12/29/58	Fever, headache, backache Vomiting, meningism Died 12/30/58	1	12/9/58	No Stool	Serum Post Mortem	1 32
			2	10/25/58			1 8
			3	11/16/58			1 32
6—C BFG 28 mos	12/22/58	Anorexia, weakness of legs High fever, pain in legs and neck, constipation Paralysis, right arm and left leg	1	—		1/10/59	<1 4
			2	10/3/58		1/10/59	1 128
			3	—	(2)	1/10/59	<1·4

TABLE 1 SUMMARY OF SUBJECTS WHO SHOWED SIGNS OF ILLNESS AFTER INGESTION OF ORAL POLIOMYELITIS VACCINE

Subject	Data on Illness		Poliovirus Data				
				Vaccine	Isolation†	Antibodies	
Assigned No., Location*, Name, Age	Onset Date	Clinical Signs	Virus Type	Feeding Date	Date of Stool	Serum Date	Titers
1—U MAG 15 mos	11/9/58	Weakness of both legs	1	—	11/11/58	No Serum	
			2	11/6/58	—		
			3	—	—		
2—U REF 19 mos	11/20/58	Monoplegia, left leg	1	—	11/30/58	5/21/59	1:32
			2	10/20/58	—	5/21/59	1:4
			3	—	—	5/21/59	<1:4
3—U BCF 4 yrs	p m 12/15/58	Paresis, right leg	1	—	12/29/58	12/29 5/11/59	1:128 1:16
			2	a m 12/15/58	—	12/29 5/11	<1:4 <1:4
			3	—	—	12/29 5/11	<1:4 <1:4
4—U SS 22 mos	12/18/58	Paraplegia	1	—	12/25/58	5/26/59	1:128
			2	12/9/58	—	5/26/59	<1:4
			3	—	—	5/26/59	1:4

5—U LPB 14 yrs	12/20/58	Quadriplegia	1	—	12/30/58	12/30 5/5/59	1:512 1:16
			2	11/14/58	—	12/30 5/5	1:32 1:1024
			3	12/4/58	—	12/30 5/5	1:512 1:64
8—U JC 4½ yrs	12/27/58	Facial paralysis	1	—	1/4/59	1/4	1:1024
			2	12/15/58	—	1/4	1:1024
			3	—	—	1/4	1:32
13—C MGD 7 mos	3/15/59	Fever, paralysis left leg	1	—	3/21/59	3/21	1:128
			2	9/26/58	—	3/21	1:32
			3	1/26/59	—	3/21	1:512
9—U MCDL 24 yrs	1/4/59	Headache, vomiting, intestinal upset Sore throat, angina pultaceous Paresis, left leg	1	12/29/58		1/20/59	1:512
			2	—		1/20/59	1:8
			3	—	(1)	1/20/59	<1:4
7—U EDTR 25 yrs	12/29/58	Fever, headache, backache Vomiting, meningism Died 12/30/58	1	12/9/58	No Stool	Serum Post Mortem	1:32
			2	10/25/58			1:8
			3	11/10/58			1:32
6—C BEG 28 mos	12/22/58	Anorexia, weakness of legs High fever, pain in legs and neck, constipation Paralysis, right arm and left leg	1	—		1/10/59	<1:4
			2	10/3/58		1/10/59	1:128
			3	—	(2)	1/10/59	<1:4

TABLE 1. SUMMARY OF SUBJECTS WHO SHOWED SIGNS OF ILLNESS AFTER INGESTION OF ORAL POLIOMYELITIS VACCINE—Continued

Subject	Data on Illness		Poliovirus Data				
				Vaccine	Isolation†	Antibodies	
Assigned No., Location*, Name, Age	Onset Date	Clinical Signs	Virus Type	Feeding Date	Date of Stool	Serum Date	Titers
12—C LCA 7 mos	2/22/59	Fever, constipation Muscular pains, difficulty in moving legs Paraplegia, paralysis of left arm	1	—	No Stool	3/21/59	<1 4
			2	11/4/58		3/21/59	<1 4
			3	1/19/59		3/21/59	1 256
10—C GLV 17 mos	1/23/59	Flaccid paralysis of right arm	1	—	(3)	2/2/59 3/4/59	<1 4 <1 4
			2	11/12/58		2/2 3/4	<1 4 <1 4
			3	1/15/59		2/2 3/4	1 32 1 32
11—U RMS 24 yrs	2/4/59	Paraplegia, paresis of intercostal and diaphragm muscles	1	1/28/59	(4)	2/11/59 5/8	1 256 1 256
			2	—		2/11 5/8	<1 4 <1 4
			3	—		2/11 5/8	<1 4 <1 4

Subjects are numbered according to date of onset of illness, but arranged in the order of presentation in text
* "U" designates Uruguay, "C" Colombia
† All stool specimens were tested for Coxsackie A virus, as well as for poliovirus
(1) Stool specimen of 1/20/59 negative for all poliovirus types
(2) Stool specimen of 1/10/59 negative for all poliovirus types.
(3) Coxsackie A-4 and poliovirus Type 3 isolated from stool specimen.
(4) Coxsackie A-4 and poliovirus Type 1 isolated from stool specimen

TABLE 2 IDENTIFICATION OF COXSACKIE A VIRUS ISOLATED FROM 2/3/59 STOOL SPECIMEN OF PATIENT NO. 10 (G. L. V.) NEUTRALIZATION TESTS: 3RD MOUSE PASSAGE, CARCASS SUSPENSION *

TEST SERIES *No*	COXSACKIE A ANTI-SERA		VIRUS DILUTION							
			10^{-4}		10^{-5}		10^{-6}		10^{-7}	
	VIRUS TYPE	DILUT	MORTALITY RATIO	AVERAGE SURVIVAL TIME (IN DAYS)	MORTALITY RATIO	AVERAGE SURVIVAL TIME (IN DAYS)	MORTALITY RATIO	AVERAGE SURVIVAL TIME (IN DAYS)	MORTALITY RATIO	AVERAGE SURVIVAL TIME (IN DAYS)
I	POOL 1, 4	1 20	0/6	10 0	†	†	0/6	10 0	—	—
	POOL 3, 5, 8, 10	1 20	8/8	2 0	8/8	4 0	8/8	3 8	—	—
	9	1 5	7/7	1 7	8/8	1 6	7/7	2 1	—	—
	13	1 5	6/6	2 0	6/6	2 0	5/5	2 2	—	—
	14	1 5	7/7	2 8	7/7	2 4	4/4	3 3	—	—
	CONTROLS NORMAL MOUSE SERUM	UNDIL	—	—	8/8	3 0	8/8	3 5	6/8	6 0
II	1	1 20	8/8	2 7	8/8	3 5	4/4	3 0	—	—
	4	1 20	0/4	10 0	0/5	10 0	0/5	10 0	—	—
	CONTROLS NORMAL MOUSE SERUM	UNDIL	—	—	6/6	3 3	4/4	3 3	7 8	3 7

* Equal volumes of anti sera and virus dilutions were mixed, incubated at 37°C for 1 hr., and inoculated subcutaneously into 1 2 day-old mice in 0 03 ml. amounts *Animals were observed for 10 days*

† Litter was eaten by mother.

TABLE 3 IDENTIFICATION OF COXSACKIE A VIRUS ISOLATED FROM 2/24/59 STOOL SPECIMEN OF PATIENT No. 11 (R. M S.)
NEUTRALIZATION TESTS: 2ND MOUSE PASSAGE, CARCASS SUSPENSION *

Test Series No	Coxsackie A Anti-Sera		Virus Dilution					
			10^{-6}		10^{-7}		10^{-8}	
	Virus Type	Dilut	Mortality Ratio	Average Survival Time (in Days)	Mortality Ratio	Average Survival Time (in Days)	Mortality Ratio	Average Survival Time (in Days)
I	Pool 1, 6, 7	1 25	8/8	3 5	6/6	5 6	—	—
	Pool 2, 3, 5, 8, 10	1 25	7/7	5 0	8/8	4 5	—	—
	4	1 20	0/8	10 0	0/5	10 0	—	—
	9	1 5	8/8	3 4	0/7	10 0	—	—
	14	1 5	8/8	3 0	8/8	4 3	—	—
	Controls Normal Mouse Serum	Undil	8/8	3 8	4/4	6 5	0/8	10 0
II	4	1 20	0/5	10 0	0/3	10 0	0/8	10 0
	9	1 5	4/4	3 0	6/6	4 4	4/6	7 4
	Controls Normal Mouse Serum	Undil	6/6	3 0	6/6	3 0	5/6	5 3

* Equal volumes of anti sera and virus dilutions were mixed, incubated at 37°C. for 1 hr, and inoculated subcutaneously into 1 2 day old mice in 0 03 ml amounts Animals were observed for 10 days

cular cuffing, one of them with additional glial clustering around shrunken neurones, and small foci of perivascular infiltration and neuronophagia in the thalamus and cerebellar nuclei. Neuronal changes were less frequent than vascular lesions, and not very extensive

Two of the seven monkeys inoculated IC (see Figs. 3-A and 3-B) showed perivascular cuffing, small scattered areas of glial proliferation, and occasional chromatolysis of motor neurons Neuronal loss was rare, with vascular round-cell infiltration the predominant pathologic change In addition to spinal cord lesions, two of the monkeys showed small inflammatory foci in the medulla, pons, red nucleus, and periaqueduct gray matter

Four of the five monkeys inoculated IS (see Figs. 4-A and 4 B) showed extensive lumbar involvement, with varying degrees of spread to the cervical enlargement. All four showed extreme

TABLE 4 SUMMARY OF RESULTS OF INOCULATION OF MONKEYS WITH COXSACKIE VIRUS ISOLATES FROM SUBJECTS NO 10 C (GLV) AND NO 11 U (RMS)

	Monkey No	Inoculation Route*			Outcome†	Histopathology			Virus Recovery	
						Lesions				
		IC	IM	IS		Brain	Cervical	Lumbar	Cervical	Lumbar
GLV Isolate	H-723	X			D-5	Not done	No	No	Yes	Yes
	H-724	X			D-5	Not done	No	No	Not	done
	H-725	X			S-6	Yes	No	No	Not	done
	H-726		X			Yes	Yes	Yes	Not	done
	H-727		X		D-15	Not done	No	No	No	No
	H-728		X		S-6	No	No	No	No	No
	H-929	X			D-11	No	Yes	Yes	No	No
	H-930	X				No	No	No	Not	done
	H-931	X				Yes	Yes	Yes	Not	done
	H-932	X				No	No	Yes	Not	done
	H-933			X		Not done	No	No	Not	done
	H-934			X		Not done	Yes	Yes	Not	done
	H-935			X		Not done	Yes	Yes	Not	done
	H-936			X		Not done	Yes	Yes	Not	done
	H-937			X		Not done	Yes	Yes	Not	done
	H-938		X			Not done	No	No	Not	done
	H-939		X		D-5	No	No	No	Yes	Yes
	H-940		X		D-10	Not done	No	No	No	No
	I-401	X	X			Not done	No	Yes	Not	done
	I-402	X	X			Not done	Yes	Yes	Not	done
	I-403	X	X			Not done	Yes	Yes	Not	done
	I-404	X	X			Not done	No	No	Not	done
RMS Isolate	I-297	X				Not done	No	No	Not	done
	I-298	X				Not done	No	No	Not	done
	I-299	X			D-7	Not done	No	No	Not	done
	I-300	X				Not done	Yes	No	Not	done
	I-301	X				Not done	No	No	Not	done
	I-302		X			Not done	No	No	Not	done
	I-303		X			Not done	No	No	Not	done
	I-304		X			Not done	No	Yes	Not	done
	I-305		X			Not done	Yes	Yes	Not	done
	I-306		X			Not done	No	No	Not	done
	J-113			X		No	No	No	Not	done
	J-114			X		Yes	Yes	Yes	Not	done
	J-115			X		Yes	Yes	Yes	Not	done
	J-116			X		Yes	Yes	Yes	Not	done
Controls‡	J-109			X		No	No	No	Not	done
	J-110			X		No	No	No	Not	done
	J-111			X		No	No	No	Not	done

* IC=Intracerebral, IM=Intramuscular, IS=Intraspinal
Doses IC & IM, separately—1.0 ml, IC & IM simultaneously—0.5 ml each route, IS—0.1 ml
Inoculum Titer 10^6 and 10^7 LD_{50} per 0.03 ml
† D=died, S=sacrificed, No.=days following inoculation that death occurred All other monkeys survived observation period
‡ Inoculated with 0.1 ml normal carcass suspension

TABLE 5 NEUTRALIZATION TITERS OF SERA FROM MONKEYS INOCULATED INTRAMUSCULARLY WITH COXSACKIE ISOLATES FROM SUBJECTS No. 10-C (GLV) AND No. 11-U (RMS)

MONKEY No	SOURCE OF ISOLATE	ANTIBODY TITERS				
		COXSACKIE A-4*		POLIOVIRUS POST-INOCULATION		
		PRE-INOCULATION	POST-INOCULATION†	TYPE 1	TYPE 2	TYPE 3
I-302	RMS	0	>1:256	<1:4	<1:4	<1:4
I-303	RMS	0	>1:256	<1:4	<1:4	<1:4
I-304	RMS	0	>1:256	<1:4	<1:4	<1:4
I-305	RMS	0	1:128	<1:4	<1:4	<1:4
I-306	RMS	0	1:128	<1:4	<1:4	<1:4
H-726	GLV	0	1:128	<1:4	<1:4	<1:4
H-938	GLV	0	1:64	<1:4	<1:4	<1:4

* Titer, against homologous strain used in inoculation, is expressed as highest dilution of serum which neutralized 100 to 500 ID_{50} of virus

† Sera collected 28 days after inoculation of monkeys

mononuclear infiltration, with some polymorphs along the needle track and spread to adjacent regions. Round-cell perivascular cuffing was common and extensive in both the cervical and lumbar areas. Neuronal loss ranged from ++++ (complete), adjacent to the track, to ++ in the opposite hemisection. The response in the cervical region ranged from severe (++++) to moderate (+) viral myelitis. As shown in Tables 6 and 7, the reaction along the track produced by IS inoculation with the isolates from Subjects 10-C and 11-U was greater than any reaction induced by IS inoculation of carcass suspension of normal suckling mice.

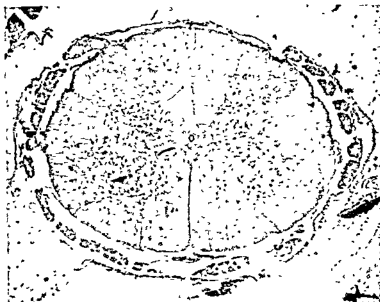
The other Coxsackie A-4 isolate (11-U), inoculated IC or IM, induced more limited spinal cord pathology, with definite neuron involvement in only one out of eight monkeys (Table 7). Round-cell infiltration was less extensive, and usually restricted to a few capillaries at more scattered levels of both the cervical and lumbar enlargements. However, IS inoculation induced numerous inflammatory foci throughout the gray matter of the spinal cord and, in two of the monkeys, lesions in the midbrain, cerebellum, pons and medulla.

Both the 10-C and the 11-U isolate induced limited polio-like spinal cord lesions, which were characteristically scattered and easily missed if an insufficient number of levels were examined. In this respect the Coxsackie A-4 isolates differed from the less attenuated polio strains, which induce more extensive and widely distributed cord lesions.

Monkey pathogenicity tests of poliovirus isolates. Table 8 summarizes the results of studies carried out in monkeys inoculated IC with the strains of Type 3 and Type 1 poliovirus recovered from Subjects 10-C and 11-U, respectively. It can be seen that none of the monkeys showed any clinical signs during the 21-day observation period. Histopathological examination of the spinal cords of monkeys inoculated with the Type 3 isolate revealed mild lesions in only one animal. Of the five monkeys inoculated with the Type 1 isolate, four showed no

FIG. 2 A Sections of Spinal Cord Enlargements of Monkey No H 726 Inoculated Intramuscularly with Coxsackie A-4 Virus Isolated from Subject No 10 C

Lumbar Enlargement



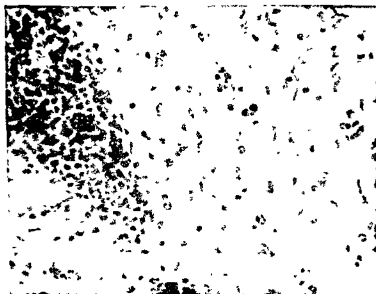


TABLE 6 RESULTS OF HISTOPATHOLOGICAL EXAMINATION OF SPINAL CORDS AND BRAINS OF MONKEYS INOCULATED WITH COXSACKIE A-4 VIRUS ISOLATED FROM SUBJECT No 10 C (GLV)

Monkey No	Inoc Route*	Cervical			Lumbar			% Lesions C+L	Remarks‡
		No of Levels Examined	% of Levels With Lesions	Range of Lesions†	No of Levels Examined	% of Levels With Lesions	Range of Lesions†		
H723	IC	7	0	0	5	0	0	0	No abnormal changes
H724	IC	4	0	0	5	0	0	0	No abnormal changes
H725	IC	16	0	0	20	5	0−+	3	Limited round-cell infiltration in lumbar Involvement of pons, medulla, red nucleus
H929	IC	39	8	0−+	35	0	0	4	*Slight neurone involvement in cervical* No definite lesions in lumbar None in brain
H930	IC	20	0	0	28	0	0	0	No abnormal changes, incl brain
H931	IC	35	6	0−++	33	9	0−++	7	Slight neurone involvement in cord Severe perivascular cuffing Involvement of pons, medulla
H932	IC	34	0	0	25	8	0−+	4	No neurone involvement Slight vascular response
H726	IM	38	8	0−++	36	9	0−++	8	Slight neurone involvement Vascular response predominant in cord Involvement of thalamus, cerebral nuclei
H727	IM	10	0	0	8	0	0	0	No abnormal changes
H728	IM	10	0	0	10	0	0	0	No abnormal changes, incl brain
H938	IM	37	0	0	38	0	0	0	No abnormal changes
H939	IM	38	0	0	6	0	0	0	No abnormal changes, incl brain
H940	IM	21	0	0	20	5	0−+	2	No neurone involvement Slight vascular response in cord

									No track, no abnormal changes
H933	IS	43	0	0	27	0	0	0	Neurone involvement away from track
H934	IS	34	3	0-+	45	33	0-+++	18	Severe cervical spread Track mostly in white matter
H935	IS	20	43	0-+++	6	16	0-++	30	
H936	IS	30	16	0-+++	38	30	0-+++	23	Severe spread contralateral to track and to cervical
H937	IS	41	5	0-++	41	34	0-++++	20	Severe spread contralateral to track and to cervical
I401	IC +IM	39	0	0	39	2	0-+	1	No neurone involvement Slight vascular response
I402	IC +IM	42	2	0-+	46	0	0	1	No neurone involvement Slight vascular response
I403	IC +IM	46	2	0-+	25	0	0	1	No neurone involvement Slight vascular response
I404	IC +IM	41	0	0	35	0	0	0	No abnormal changes

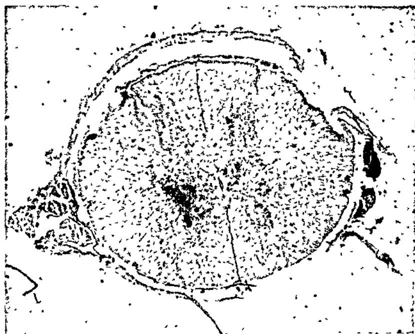
* IC=intracerebral, IM=intramuscular, IS=intraspinal

† Least to most severe ‡ Including brain findings where brain was examined

All inoculated with 3rd suckling mouse passage of GLV stool, 10% carcass suspensions Inocula IC, IM—1 0 ml, IS—0 1 ml, IC and IM—0 5 ml

FIG 2 B Sections of Spinal Cord Enlargements of Monkey No H-726 Inoculated Intramuscularly with Coxsackie A-4 Virus Isolated from Subject No 10 C

Cervico Thoracic Enlargement



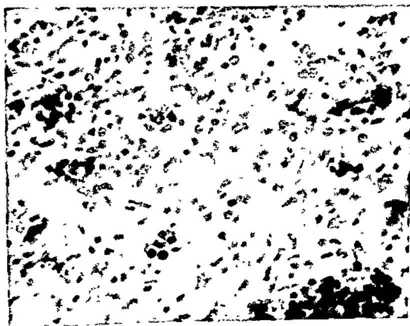
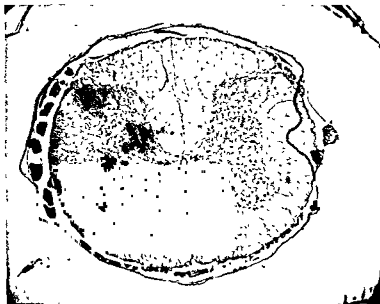


Fig 3-A. Sections of Spinal Cord Enlargements of Monkey No H 931 Inoculated Intracerebrally with Coxsackie A-4 Virus Isolated from Subject No 10 C

Lumbar Enlargement



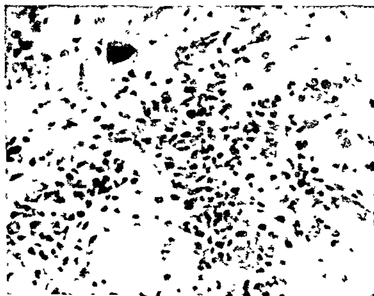
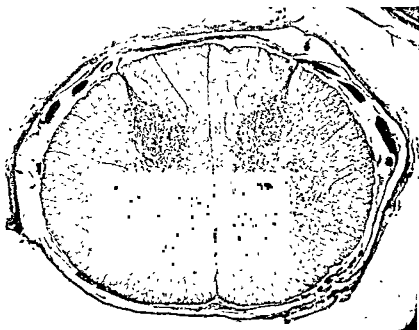


Fig. 3 B. Sections of Spinal Cord Enlargements of Monkey No H-931 Inoculated Intracerebrally with Coxsackie A 4 Virus Isolated from Subject No. 10 C

Cervical Enlargement



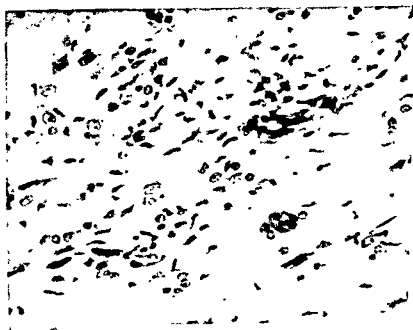


Fig. 4-A. Sections of Spinal Cord Enlargements of Monkey No. H 937 Inoculated Intraspinally with Coxsackie A-4 Virus Isolated from Subject No. 10-C

Lumbar Enlargement



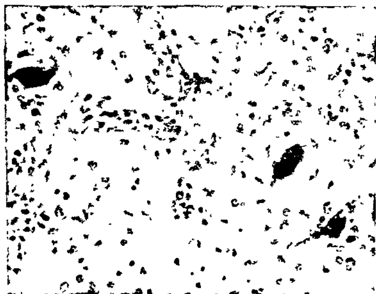
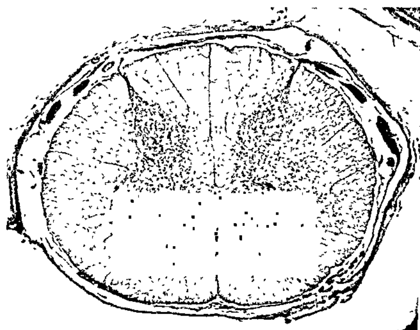


Fig 3-B Sections of Spinal Cord Enlargements of Monkey No H 931 Inoculated Intracerebrally with Coxsackie A-4 Virus Isolated from Subject No 10 C

Cervical Enlargement



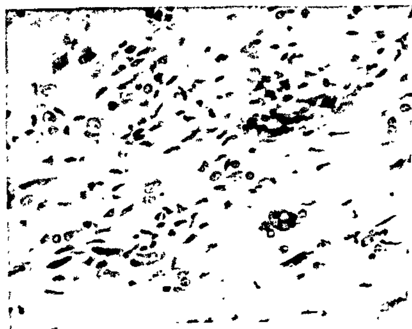


TABLE 7 RESULTS OF HISTOPATHOLOGICAL EXAMINATION OF *SPINAL CORDS* AND BRAINS OF MONKEYS INOCULATED WITH COXSACKIE A 4 VIRUS ISOLATED FROM SUBJECT NO 11 U (RMS)

Monkey No	Inoc Route*	Cervical: No of Levels Examined	Cervical: % of Levels With Lesions	Cervical: Range of Lesions†	Lumbar: No of Levels Examined	Lumbar: % of Levels With Lesions	Lumbar: Range of Lesions†	% Lesions C+L	Remarks (Including Brain Findings Where Brain Was Examined)
I297	IC	37	0	0	48	0	0	0	No abnormal changes
I298	IC	29	0	0	49	0	0	0	No abnormal changes
I299	IC	25	0	0	32	0	0	0	No abnormal changes
I300	IC	16	6	0 − +	44	0	0	3	No neurone involvement, slight vascular response
I301	IC	50	0	0	59	0	0	0	Meningitis
I302	IM	38	0	0	50	0	0	0	No abnormal changes
I303	IM	42	0	0	48	0	0	0	No abnormal changes
I304	IM	53	0	0	51	8	0 − +	4	No neurone involvement, moderate vascular response
I305	IM	41	2	0 − + +	41	2	0 − + +	2	*Limited but multiple* vascular and neurone involvement in cord
I306	IM	50	0	0	42	0	0	0	No abnormal changes
J113	IS	36	0	0	38	0	0	0	No lesions, no track
J114	IS	53	8	0 − + +	64	62	0 − + + +	35	Extensive spread of lumbar lesions to many levels Foci in medulla, pons, substantia nigra
J115	IS	46	52	0 − + +	52	75	0 − + + +	64	Numerous foci in pons, medulla, midbrain, dentate nuclei, thalamus
J116	IS	38	5	0 − +	42	15	0 − + +	20	Few lesions in midbrain
CONTROLS									
J109	IS	42	0	0	36	0	0	0	Needle track No other lesions
J110	IS	40	0	0	44	0	0	0	Needle track No other lesions
J111	IS	47	0	0	52	0	0	0	Needle track No other lesions

* IC=intracerebral; IM=intramuscular, IS=intraspinal
† Least to most severe
Controls inoculated with 0 1 ml normal carcass suspension, all others with 2nd suckling mouse passage of RMS stool, 10% carcass suspensions, 1 0 ml IC and IM; 0 1 ml IS.

Fig 4 B Sections of Spinal Cord Enlargements of Monkey No H-937 Inoculated Intraspinally with Coxsackie A-4 Virus Isolated from Subject No 10 C

Cervical Enlargement



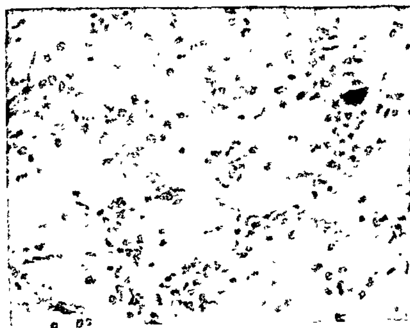


TABLE 9 SUMMARY OF RESULTS OF SIMULTANEOUS INOCULATION OF MONKEYS WITH TYPE 3 POLIOVIRUS AND COXSACKIE A 4 STRAINS ISOLATED FROM THE SAME STOOL SPECIMEN OF SUBJECT NO 10 C (GLV)

Monkeys were inoculated first IC with 1 0 ml Type 3 poliovirus 3rd monkey-kidney-tissue-culture passage with virus content of 10^7 $TCID_{50}$, and 5 days later IM with 1 0 ml of 10% carcass suspension of the 3rd suckling mouse passage of Coxsackie A-4, with virus content of 10^7 mouse LD_{50}.

MONKEY No	CLINICAL SIGNS	PATHOLOGY
I-708	None	Discrete perivascular infiltrations in medulla and spinal cord
I-709	None	None
I-710	Found dead, choked with the chain*	None Post mortem changes
I-711	None	None
I-712	None	Numerous lesions in spinal cord, medulla, pons, mid-brain, dentate nuclei, cerebral cortex

* Found dead 9 days after inoculation No clinical manifestation before death

lesions, the fifth showed numerous scattered lesions of mild to moderate degree throughout the spinal cord These findings are essentially comparable to those induced in monkeys by the Type 1 and Type 3 poliovirus vaccines which had been ingested by the subjects

The results of dual infection of monkeys with Coxsackie A-4 and poliovirus Type 3 are presented in Table 9 As can be seen, none of the monkeys showed any clinical manifestation, even the one showing maximum spread of lesions in the central nervous system

Comments

Chart 1 summarizes the histories of the seven subjects who ingested only Type 2, or both Type 2 and Type 3, poliovirus vaccine, and from whose stool specimens only Type 1 poliovirus was isolated It is difficult to believe that the vaccine virus had a direct etiologic relationship with the illnesses of any of these subjects Furthermore, six of these subjects lived in Montevideo, and we wish to emphasize that Type 1 poliovirus was known to be prevalent in the Montevideo metropolitan area prior to the initiation of the mass vaccination program there Since none of them had been fed Type 1 virus, the summary presentation of data on these subjects will suffice, and we shall proceed to discuss the remaining six cases individually and in detail

Subject 9 U (MCDL)—The stool specimen obtained from this patient, taken 22 days after ingestion of Type 1 vaccine and 16 days after onset of her illness, yielded no virus in several isolation attempts in MK and HeLa cell cultures or in suckling mice A brief history of this patient's illness is presented in Chart 2.

Since the clinical manifestations observed in this patient are seen in patients with other infectious diseases such as ECHO and Coxsackie virus infections, and in certain non-infectious diseases, no definitive etiologic diagnosis could be made without supporting laboratory studies Because excretion of Type 1 poliovirus, both in natural poliomyelitis and in infections induced by the vaccine strain, can continue for prolonged periods, failure to find poliovirus in a stool sample obtained 16 days after the onset of symptoms argues strongly against a diagnosis of

TABLE 8. SUMMARY OF RESULTS OF INTRACEREBRAL INOCULATION OF MONKEYS WITH TYPE 3 POLIOVIRUS ISOLATED FROM STOOL SPECIMEN OF SUBJECT No. 10 C (GLV) AND WITH TYPE 1 POLIOVIRUS ISOLATED FROM SPECIMEN OF SUBJECT No 11 U (RMS)

Animals were observed for 21 days, then sacrificed

Monkey No	Clinical Signs	Cervical			Lumbar			Remarks
		No of Levels Examined	% of Levels with Lesions	Range of Lesions (Least to Most)	No of Levels Examined	% of Levels with Lesions	Range of Lesions (Least to Most)	
MONKEYS INOCULATED WITH TYPE 3*								
I-449	None	42	0	0	26	0	0	No abnormal changes
I 450	None	28	0	0	43	0	0	No abnormal changes
I 451	None	39	0	0	39	0	0	No abnormal changes
I 452	None	42	0	0	47	0	0	No abnormal changes
I-453	None	52	8	0 ++	47	0	0	Neurones not involved Moderate vascular response
MONKEYS INOCULATED WITH TYPE 1†								
I-713	None	66	0	0	66	0	0	No abnormal changes
I-714	None	63	0	0	60	0	0	No abnormal changes
I-715	Found dead 10th day ‡	57	0	0	51	0	0	No abnormal changes
I-716	None	60	63	0-++	60	66	0-+++	Some neuronal involvement Multiple foci
I-717	None	90	0	0	80	0	0	No abnormal changes.

* Each animal received a 1.0 ml dose of 3rd monkey kidney-tissue culture passage, containing 10^7 $TCID_{50}$.
† Each animal received a 1.0 ml dose of 2nd monkey kidney-tissue culture passage, containing 10^7 $TCID_{50}$.
‡ This monkey showed no clinical signs before death

CHART 2. SUBJECT No 9—U—MCDL— 21 YEARS—FEMALE

History

29 December, 1958—Fed poliovirus Type 1 vaccine
4 January, 1959—Headache, vomiting, intestinal upset
10 January —Sore throat, angina pultaceous
17 January —Pain in gastrocnemius muscles of left leg, and patient drags left foot when walking
Examination revealed paresis of peronaeus muscles of left side Reflexes normal
18 January —Slight stiff neck
20 January —Stool and blood specimens collected
28 January —Full recovery

STOOL SPECIMEN NEGATIVE FOR ALL POLIOVIRUS TYPES AND COXSACKIE

Poliovirus Neutralizing Antibodies in Serum

TYPE 1	TYPE 2	TYPE 3
1 512	1 8	<1 4

poliomyelitis, and the same conclusion is suggested by the serologic findings and the patient's rapid recovery Had earlier and additional stool and blood samples been available, a more complete and more satisfactory diagnostic study could have been made To help answer any question as to the validity of the results of the stool specimen examination, because of the possibility of inadequate refrigeration or other mishap during transportation, it should be pointed out that this specimen was received at Lederle Laboratories in the same package with three other fecal samples, and from each of the other specimens strains of poliovirus were isolated

All available information about Subject 9-U suggests that her illness was caused by some agent other than poliovirus It is significant to note that the original diagnosis of poliomyelitis was withdrawn, and the Poliomyelitis Commission of Montevideo recorded the final diagnosis as *polyneuritis*.

CHART 3. SUBJECT No 7—U—EDTR— 25 YEARS—MALE

History

Previous History —2-year history of rheumatic disease, enlarged heart, arthritic involvement of left shoulder Was under cortisone treatment Ordered by physician to avoid sports and strenuous exercise For past 3 months had severe headaches and dyspnea which forced him intermittently to stop his work as bookkeeper A day or 2 prior to onset of illness, had spent the day on the beach where he played soccer
25 October, 1958—Fed poliovirus Type 2 vaccine
16 November —Fed poliovirus Type 3 vaccine
9 December —Fed poliovirus Type 1 vaccine
29 December —Fever, headache, backache
30 December —Symptoms of previous day, and vomiting, meningism
Reflexes of legs hyperactive
Paresis of left arm.
About midnight patient became dyspneic and cyanotic and was put in respirator
Two hours later, died
Only specimens obtained were cerebrospinal fluid a few hours before death and a post-mortem blood sample.
Family refused autopsy

NO STOOL SPECIMEN

Poliovirus Neutralizing Antibodies in Post-Mortem Serum

TYPE 1	TYPE 2	TYPE 3
1 32	1 8	1.32

Results of Cerebrospinal Fluid Examination

Glucose	Normal
Chlorides	Normal
Protein	20 mg /100 ml
Cells per cmm	130 (60% monocytes)

CHART 1. SUMMARY OF 7 SUBJECTS WITH SIGNS OF NEUROLOGICAL ILLNESS AFTER INGESTION OF TYPE 2 OR TYPE 3 ORAL POLIOMYELITIS VACCINE FROM WHOSE STOOL SPECIMENS ONLY TYPE 1 POLIOVIRUS WAS ISOLATED

Subject	Data on Illness		Poliovirus Data				
	Onset Date	Clinical Signs	Virus Type	Vaccine	Isolation	Antibodies	
				Feeding Date	Stool Date	Serum Date	Titers
1-U MAG 15 mo	11/ 9/58	Weakness of both legs	1	—	11/11/58	NO SERUM	
			2	11/ 6/58	—		
			3	—	—		
2-U REF 19 mo	11/20/58	Monoplegia of left leg	1	—	11/30/58	5/21/59	1.32
			2	10/20/58	—	5/21/59	1 4
			3	—	—	5/21/59	<1 4
3-U BCF 4 yr	p m 12/15/58	Paresis of right leg	1	—	12/29/58	12/29/58	1 128
			2	a m 12/15/58	—	12/29/58	<1 4
			3	—	—	12/29/58	<1 4
4-U SS 22 mo	12/18/58	Paraplegia	1	—	12/25/58	5/26/59	1 128
			2	12/ 9/58	—	5/26/59	<1 4
			3	—	—	5/26/59	1 8
5-U LPB 14 yr	12/20/58	Quadriplegia	1	—	12/30/58	12/30/58	1:512
			2	11/14/58	—	12/30/58	1:32
			3	12/ 4/58	—	12/30/58	1.512
8-U JC 4½ yr	12/27/58	Facial paralysis	1	—	1/ 4/59	1/ 4/59	1 1024
			2	12/15/58	—	1/ 4/59	1 1024
			3	—	—	1/ 4/59	1.32
13-C MGD 7 mo.	3/15/59	Fever, paralysis of left leg	1	—	3/21/59	3/21/59	1 128
			2	9/26/58	—	3/21/59	1.32
			3	1/26/59	—	3/21/59	1.512

present in the subject's serum 33 days after onset

The symptoms presented by this subject are very suggestive of clinical paralytic poliomyelitis, and the presence in the serum of poliovirus antibodies only for Type 3 suggests that it was this type that was responsible for the illness. However, Type 3 oral vaccine was fed to this subject 34 days before onset of her illness, and an incubation period of that duration is so far beyond the range as determined by Sartwell*, that either a wild Type 3 strain or some other etiologic agent must be seriously considered as the cause of illness.

The history of Subject 10-C (GLV) is presented in Chart 6 Clinical signs in this case constitute the common syndrome seen in patients with enterovirus infections, including paralytic and nonparalytic poliomyelitis.

Chart 7 presents the clinical history of Subject

CHART 5 SUBJECT NO 12—C—LCA—
7 MONTHS—FEMALE

History

4 November, 1958	—Fed poliovirus Type 2 vaccine
19 January, 1959	—Fed poliovirus Type 3 vaccine
22 February	—High fever, continuing for 6 days Constipation Cold-like symptoms
24 February	—Muscular pains
28 February	—Paraplegia paralysis of left arm
21 March	—Stool and blood specimens collected from patient and household contacts *
15 April	—Paralysis of arm improving, with only slight shoulder involvement remaining

NO STOOL SPECIMEN

Poliovirus Neutralizing Antibodies in Serum

TYPE 1	TYPE 2	TYPE 3
<1 4	<1 4	1 256

* Only blood serum specimen from patient was received at Lederle Laboratories

11 U (RMS). In some respects her symptoms are similar to those of Subject 10 C, but the clinical history of RMS is confusing Her rapid improvement 24 hours after showing "paraplegia" and difficulty with respiration, presumably suspected of being bulbar in origin, suggests very strongly that certain of her neurological symptoms should be accepted with some reservations According to a report dated 2 June, she gave birth to a normal child shortly after leaving the hospital and although she still has some leg weakness, her full recovery is anticipated The least questionable of the symptoms listed in her clinical history thus seem to be the fever headache, vomiting and muscular weakness which are consistent with enterovirus infection and this is further suggested by the findings in her cerebro spinal fluid

The recovery of polioviruses in conjunction with Coxsackie A-4 in stool samples collected from both Subjects 10-C and 11-U suggests the possibility that in these two cases either one or both of these agents might have been involved in the illnesses This is further indicated by the capacity of serum specimens collected sometime after the onset of their illnesses to neutralize both the poliovirus and Coxsackie A-4 virus isolated from their respective stool specimens

From the available laboratory information it is not possible to rule out either agent from responsibility for the illnesses of these two patients However the excretion of poliovirus which was demonstrated in the stool specimens of both patients is known to occur frequently when susceptible individuals are successfully vaccinated with live oral poliovirus vaccine, and the finding of poliovirus antibodies in their sera is an expected consequence of such infection

We have no information on the prevalence of Coxsackie virus infections in Colombia and Uruguay, but there is reason to believe that the incidence of such infections there is similar to that reported from several other parts of the world Hence the Coxsackie A-4 viruses found in their intestinal tracts could be considered so-called "fellow travelers" with no etiologic role in the clinical illnesses of the patients

Coxsackie A 4 is known to be one of the virus types frequently associated with the herpangina syndrome, but it also has been reported to be etiologically related to illness associated with neurological signs. In Brazil, Paulo de Goes

Concerning Subject 7-U (EDTR), the interpretation of the clinical picture, which is summarized in Chart 3, lacking as it does both adequate laboratory information and necropsy studies, makes speculative the etiology of his fatal illness The Poliomyelitis Commission of Montevideo made a clinical analysis of this case and concluded that poliovirus was not involved in the illness, and withdrew the case from the official list of poliomyelitis cases

The next subject to be discussed is 6-C (BEG) As shown in Table 1 and in Chart 4, this subject was fed Type 2 poliovirus vaccine in Medellin on 3 October and 80 days later, on 22 December, showed signs of illness very suggestive of paralytic poliomyelitis However, stool specimens collected on 10 January 1959, 19 days after onset of illness, from the patient and her household contacts yielded no virus of any type

As can be seen the patient's serum contained antibodies only to Type 2 poliovirus, presumably due to ingestion of Type 2 vaccine more than 11 weeks before the collection of the first blood specimen, which occurred three weeks after onset of her illness The failure to isolate a virus from the patient's stool, and the finding of a Type 2 poliovirus antibody titer of 1 128 in her serum sample, do not rule out the possibility of an infection from a wild strain of Type 2 virus However, we believe that the Type 2 poliovirus vaccine strain can be ruled out as the cause of her illness because it was fed 80 days before onset.

Based on a statistical analysis of the data from several epidemiological studies, including the explosive Broadstairs milk-borne outbreak in England, Casey's rural epidemic, and a series of post tonsillectomy cases, Sartwell has established a paralytic poliomyelitis incubation period range of 7 to 20 days, with a median of 12 days[6] He also *found that the onset of paralysis in* chimpanzees and cynomolgus monkeys after ingestion of virulent strains of poliovirus was in good agreement with the median incubation period in man

In order *to conclude* with a discussion of the other two remaining subjects, we shall next take up Subject 12 C (LCA) whose clinical history appears in Chart 5. It is difficult to discuss this case without laboratory information on stool and serum specimens collected soon after the onset of illness Apparently the medical staff engaged with the trial in this area did no know of the case until almost a month after onset, and all stool samples then collected from *the patient and her household contacts*, as well as the serum specimens of the contacts, failed to reach Lederle Laboratories Thus the only laboratory information obtainable was that concerning the poliovirus neutralizing antibodies

CHART 4 SUBJECT NO. 6—C—BEB—28 MONTHS—FEMALE

History

3 October,	1958—Fed *poliovirus Type 2 vaccine*
22 December	—Anorexia, weakness of legs
26 December	—High fever, pains in legs and neck, constipation
29 December	—Paralysis of right arm and left leg Temperature down. Incontinence
	—Cerebrospinal fluid examination:
	Protein 61 mg /100 ml
	Glucose 66 mg/100 ml
	Cells per cemm
	Polymorphonuclears 39
	Lymphocytes 54
10 January,	1959—Blood and stool specimens collected from patient and household contacts
15 April	—Paralysis of left leg had totally regressed

STOOL SPECIMENS FROM SUBJECT AND CONTACTS NEGATIVE FOR ALL POLIOVIRUS TYPES AND COXSACKIE

Poliovirus Neutralizing Antibodies in Sera of Subject and Contacts

NAME	AGE	TYPE 1	TYPE 2	TYPE 3
BEG	28 mo	<1 4	1 128	<1 4
LGG	47 yr	1 512	>1,1024	1 256
BRG	40 yr	1 256	>1 1024	1 256
LG	18 yr	1 128	1 512	1 64

CHART 7. SUBJECT NO 11—U—RMS—24 YEARS—FEMALE, 7 MOS. PREGNANT

History

20 January, 1959—Fed poliovirus Type 1 vaccine

4-7 February —Fever, vomiting, headache, loss of muscular strength of arms and legs

10 February —Hospitalized

Tachycardia, cyanosis, questionable light paresis of diaphragm and intercostal muscles

Light stiff neck, muscular pains in scapular region, paresis of arms

Paraparesis, but patient could move her feet and was able to walk on arrival at hospital

Kernig negative Radius-cubital reflex absent Patellar reflex absent Achilles tendon reflex present Abdominal reflex absent Sensibility normal Angina pultaceous

Put in iron lung

Cerebro-spinal fluid examination

Pandy—weak positive

Glucose—50 mg /100 ml

Protein—50 mg /100 ml

Cells per ccmm—60

11 February —Muscular strength recovered

Reflexes normal

Blood specimen collected

Respiratory difficulty disappears, removed from respirator.

Left hospital a few days later greatly improved

24 February —Stool specimen collected

5 May —Normal infant delivered by Caesarian section

8 May —Second blood specimen collected

2 June —Can walk alone Full recovery is expected

Virus Isolation from Stool Specimen

DATE OF SPECIMEN	POLIOVIRUS ISOLATED	COXSACKIE ISOLATED
2/24/59	Type 1	A-4

Neutralizing Antibodies in Serum

DATE OF SERUM	POLIOVIRUS			COXSACKIE A
	TYPE 1	TYPE 2	TYPE 3	TYPE 4
2/11	1 256	<1 4	<1 4	1 32
5/8	1 256	<1 4	<1 4	1 64

reported three instances of the isolation of Coxsackie A-4 one case with a clinical diagnosis of aseptic meningitis, one diagnosed as meningoencephalitis, and a third patient with a history of acute illness and in all three cases a regressive paralysis was present.[7] In the first two cases the viral agent was isolated both from cerebrospinal fluid and from feces, and in the last case from feces only

In addition, Howitt has reported the isolation of Coxsackie A-4 virus from nervous tissue of five fatal cases, three of which were diagnosed clinically as poliomyelitis[8] From one of these cases poliomyelitis and Coxsackie viruses were recovered simultaneously from the spinal cord

N F Stanley and D C Dorman of Australia reported the isolation of Coxsackie A-4 from a case clinically diagnosed as paralytic poliomyelitis with regressive paralysis, where attempts to recover poliovirus by monkey inoculation were unsuccessful[9] Most recently it has been reported that Dr A R Fodor of the Communicable Disease Center has isolated Coxsackie A-9 virus from an acute phase blood specimen taken from a patient in Georgia who was gravely paralyzed "from the neck down" but made a dramatic recovery which enabled him subsequently to engage in school athletics The report states, 'Neutralization tests on acute and convalescent sera showed an increase in titer to

Chart 6 Subject No 10–C–GLV–17 Months—Male

History

12 November, 1958	Fed poliovirus Type 2 vaccine
15 January, 1959	Fed poliovirus Type 3 vaccine
23 January,	Fever, sore throat, diarrhea
1 Febuary,	Flaccid paralysis of right arm
2 & 3 February,	Stool and blood specimens collected from patient and household contacts
1 March,	Second stool and blood specimens collected from patient and some household contacts
15 May,	Report on follow-up of patient stated that although he has received no physiotherapy, there has been some regression of paralysis. Second specimens collected from some household contacts

Studies of Stool and Serum Specimens from Patient and Contacts

Name	Age and Sex	Poliovirus Type 3 Feeding Date	Virus Isolation: Date of Stool	Virus Isolation: Polio Type 3 $TCID_{50}$/Gr	Virus Isolation: Cox A Type 4 LD_{50}/Gr	Neutralizing Antibodies: Date of Serum	Poliovirus Type 1	Poliovirus Type 2	Poliovirus Type 3	Coxsack A Type 4
GLV*	17 mo M	1/15	2/2 3/4	1 000 0	35 000 300	2/2 3/4	<1:4 <1:4	<1:4 <1:4	1:32 1:64	1:32 >1:256
AAV*	3 yrs M	1/15	2/2	Traces	Negative	2/3 3/4	1:64 1:128	1:8 1:8	>1:1024 >1:1024	1:128 >1:256
GAV*	6 yrs M	1/15	2/2	Traces	Negative	2/3 5/15	1:128 1:64	1:128 1:128	>1:1024 1:512	1:64 1:64
JJV*	7 yrs M	1/15	2/2	Negative	Negative	2/3 5/15	1:256 1:1024	1:1024 1:1024	<1:4 1:128	1:64 1:128
JMV†	31 yrs M	None	2/2	Traces	Negative	2/3	1:32	1:32	1:32	1:32
MMV	25 yrs F	None	2/2	Traces	Negative	2/3 5/15	1:128 1:256	1:64 1:32	>1:1024 1:256	1:32 1:32

* Also fed Type 2 vaccine
† Not available for second bleeding

panied by clinical manifestations further studies with these Coxsackie strains are in progress

The demonstration of the neurotropism of Coxsackie A-4 isolates in monkeys as reported here underscores once more the necessity of further investigation of the ecology of the enterovirus groups, previously emphasized by Dalldorf[14] and Habel[15]

SUMMARY

The clinical histories and laboratory findings are summarized for 13 subjects who developed significant or suspicious illness during the field trials of oral poliovirus vaccine in Uruguay and Colombia

Causal relationship between the illness and the vaccine viruses is ruled out in two cases from whose stool no virus was isolated or from whom no specimen was received for isolation studies on the basis that the intervals between vaccine feeding and onset of illness were well outside the established range of the incubation period for paralytic poliomyelitis in man

In two cases the clinical data failed to support a diagnosis of poliomyelitis and the cases were withdrawn from the official list after careful review by the local medical authorities

Six cases in Montevideo, where a Type 1 polio virus epidemic was in progress, yielded Type 1 virus before they had been fed the Type 1 vaccine One Colombia subject also was found to harbor Type 1 virus prior to ingestion of Type 1 vaccine

In the only two instances in which the subject's stool yielded the same type of poliovirus as that recently received in the oral vaccine, strains of Coxsackie A-4 virus were isolated from the same stool samples One of these cases, in Uruguay, involved a Type 1 poliovirus-Coxsackie mixture, and the other in Colombia, a Type 3 poliovirus-Coxsackie combination A study of the poliovirus and Coxsackie virus isolates individually and in combination in monkeys revealed neurotropic activity in the Coxsackie A-4 virus which indicates a strong likelihood that the Coxsackie virus was etiologically related to the disease and regressive paralysis observed in these two subjects

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Coxsackie A-9 of from less than 1:8 to 1:32. Throat washings, two stools and a cerebrospinal fluid specimen, all obtained early in the course of the illness, were negative for any virus growth. Complement-fixation tests for the three poliovirus types were negative in both the acute and convalescent sera"[10]

Neurotropism has been demonstrated in Coxsackie A, Types 1, 14[11] and especially Type 7,[12, 13] and their frequent association with polioviruses in epidemic paralytic poliomyelitis[14] has led some investigators to suspect that other members of the Coxsackie A group of viruses may also be able to invade the human CNS Based on this assumption, it would not be difficult to conclude that, in some dual intestinal-tract infections with poliovirus and Coxsackie A, neurological symptoms could result from Coxsackie A virus invasion of the CNS without its penetration by poliovirus

Furthermore paralysis in monkeys exposed to dual infection with poliovirus and Coxsackie A has been experimentally induced by Dalldorf.[15] For these experiments he used Sabin's attenuated strain of Type 1 poliovirus and Coxsackie A, Type 7 or Type 14. In separate experiments, both the poliovirus strain and the Coxsackie A strains had previously been shown not to cause paralysis in monkeys But the poliovirus strain inoculated intracerebrally produced neuronal degeneration The Coxsackie strains when inoculated simultaneously by the IC and IM routes produced lesions of the anterior horns of the spinal cord The results of these experiments suggested to Dalldorf the possibility that paralysis developing in human beings suffering from dual infection with poliovirus and Coxsackie A might be due to the summation of the neuron destruction by the two viral agents Accordingly, Dalldorf has called for caution in the use of attenuated poliovirus vaccines where Coxsackie A virus might be encountered

The experimental findings presented here in the study of the polioviruses and the strains of Coxsackie A-4 isolated from the fecal specimens of the two vaccinated subjects showing polio-like neuro-symptoms prompt the following observations:

(1) The Type 1 and Type 3 polioviruses that were isolated from the two patients who had been fed homologous monovalent oral vaccines were shown to have the same degree of neurotropism as that possessed by the original strains used in the preparation of the vaccines. Since both of these attenuated vaccine strains now have been fed to more than 100,000 susceptible individuals, many of them triple negatives, without harmful effect, it seems most unlikely that either strain could have been responsible for the nervous manifestations observed in either of these subjects.

(2) To date there have been only two instances in which the same type of virus fed in the vaccine was recovered from the feces of a patient showing nervous symptoms. In both of these cases, Coxsackie A-4 virus was recovered simultaneously with the poliovirus Coxsackie A 4 virus has been recovered by other investigators from patients manifesting polio-like syndromes who were in no way associated with the administration of live virus vaccines or with field strains of poliovirus

(3) The Coxsackie A-4 strains recovered from these patients have both been proved to have the ability to produce polio-like lesions in the anterior horns of the spinal cord when inoculated into cynomolgus monkeys, whether by the IC, IM, or IS route. This fact strongly suggests the responsibility of these Coxsackie strains for the clinical manifestations observed in the patients

(4) Finally, epidemiological information regarding the other enteroviruses including the Coxsackie Group A, indicates that their seasonal and geographic distribution and their modes of transmission are essentially the same as those of the polioviruses, and the two are constantly found in association with each other It might therefore be expected that if these viruses operate synergistically to cause paralysis, the conditions most favorable to the simultaneous transmission of polioviruses and Coxsackie viruses would be associated also with the greatest frequency of paralytic poliomyelitis However, what is known abou the epidemiology of paralytic poliomyelitis is not in accord with this hypothesis

It is worth noting that monkeys inoculated simultaneously with Type 3 poliovirus and Coxsackie A-4 isolated from Subject 10-C showed no clinical manifestations, even though one monkey showed extensive lesions throughout the central nervous system. Because of the remarkable nature and distribution of these lesions, unaccom-

DISCUSSION

CHAIRMAN ARMSTRONG The paper presented by Dr Roca García is now open for discussion

DR. COX: As you may know, whenever we apply for a license for a biological product, we must tell everything that we know about the product—the good, as well as perhaps the not-so-good A rumor that during some of our trials we did not have proper surveillance, recently came to our attention Accordingly we have redoubled our efforts in every possible respect, so that we might know exactly what was going on, insofar as that is possible, and we have repeatedly insisted that all clinically suspect cases be brought to our attention as soon as possible The fact that some of the materials repeatedly requested, such as stool specimens and blood samples, were not delivered to us sooner is not due to a lack of effort on our part at Pearl River When you stop and consider that during the feeding of at least two million doses of live virus polio-vaccine only 13 cases of suspected poliomyelitis occurred, and that of these 13 only two cases were of the same sero type as the virus fed, then you properly realize that this is not serious trouble at all Furthermore, Dr Roca García has just covered adequately all the suspect cases that have come to our knowledge, and we feel confident that we have a clean bill of health

To my knowledge, this is the first frank and detailed follow up of any of the large groups vaccinated in the field that have been reported on in this session

Yesterday I noted that 15 cases of polio were reported in Mexico City during the feeding of approximately 100,000 people Three of the 15 cases apparently were followed up and dismissed as vaccine breaks I am not too sure that, by our criteria, we would consider this as entirely satisfactory As you know, the remaining 12 cases likewise should be followed up and fully accounted for, just as we have tried to do in our studies

Now, yesterday the Russian workers stated that they had had no troubles whatsoever, but when you are dealing with millions of people, as they apparently are, I doubt that such a flat statement can be made without more adequate documentary proof Everyone here recognizes that it would be difficult to feed two million people that many doses of distilled water without some possible untoward occurrences that would have to be looked into

As Dr Fred Soper has pointed out, it was not until approximately 1,600,000 doses of yellow fever vaccine had been administered that some troubles were encountered in the field No one can guarantee anything in this world to be perfect No one can guarantee that all of us will get home from this trip safely, as we hope we will

Frankly, from my experience in the field of biologics, I know that we have never before encountered so little trouble with any other biological preparation, even though we have never before worked on such a large scale in the field I believe that I speak with some authority, because I believe that our group has had about as much experience with either killed vaccines or living virus vaccines as almost any other group that could be named at the present time

As I have said before, it is our duty and responsibility to tell everything we know If we did not do that, we would be remiss Some people may sit here shaking their heads and perhaps saying "Oh, this is a terrible thing, you are in trouble" But let us wait and see what the other workers do about producing the same follow-up evidence that we have I welcome your attack

CHAIRMAN ARMSTRONG I am sure that if we all follow that perceptive approach—being open, frank, and honest—all these problems are solvable

DR DICK I think these findings indicate one of the most important things in studies with live virus vaccines that is, careful surveillance

Now, this has to be done in a very thorough way; and one criticism I would raise of these studies is the interval in some individuals from

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DISCUSSION

CHAIRMAN ARMSTRONG: The paper presented by Dr Roca-García is now open for discussion

DR COX: As you may know, whenever we apply for a license for a biological product, we must tell everything that we know about the product—the good, as well as perhaps the not-so-good A rumor that during some of our trials we did not have proper surveillance, recently came to our attention Accordingly we have re doubled our efforts in every possible respect, so that we might know exactly what was going on, insofar as that is possible, and we have repeatedly insisted that all clinically suspect cases be brought to our attention as soon as pos sible The fact that some of the materials repeatedly requested, such as stool specimens and blood samples, were not delivered to us sooner is not due to a lack of effort on our part at Pearl River When you stop and consider that during the feeding of at least two million doses of live virus polio vaccine only 13 cases of suspected poliomyelitis occurred, and that of these 13 only two cases were of the same sero type as the virus fed, then you properly realize that this is not serious trouble at all Furthermore, Dr Roca Garcia has just covered adequately all the suspect cases that have come to our knowl edge, and we feel confident that we have a clean bill of health

To my knowledge, this is the first frank and detailed follow up of any of the large groups vaccinated in the field that have been reported on in this session

Yesterday I noted that 15 cases of polio were reported in Mexico City during the feeding of approximately 100 000 people Three of the 15 cases apparently were followed up and dismissed as vaccine breaks I am not too sure that, by our criteria, we would consider this as entirely satisfactory As you know, the remaining 12 cases likewise should be followed up and fully accounted for, just as we have tried to do in our studies

Now, yesterday the Russian workers stated that they had had no troubles whatsoever, but when you are dealing with millions of people, as they apparently are, I doubt that such a flat statement can be made without more adequate documentary proof Everyone here recognizes that it would be difficult to feed two million people that many doses of distilled water without some possible untoward occurrences that would have to be looked into

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As I have said before, it is our duty and responsibility to tell everything we know If we did not do that, we would be remiss Some people may sit here shaking their heads and perhaps saying "Oh, this is a terrible thing, you are in trouble" But let us wait and see what the other workers do about producing the same follow up evidence that we have I welcome your attack

CHAIRMAN ARMSTRONG: I am sure that if we all follow that perceptive approach—being open, frank, and honest—all these problems are solvable

DR DICK: I think these findings indicate one of the most important things in studies with live virus vaccines, that is, careful surveillance

Now, this has to be done in a very thorough way, and one criticism I would raise of these studies is the interval in some individuals from

the time of onset to the time of taking the specimens.

Now, we all know that when a child becomes paralyzed, he comes into the hospital, and you may isolate immediately the type of virus, but as the child remains in the hospital for some time he may pick up another virus, and I feel that other interpretations of the data might be made because of the long delay which exists, up to 20 days in some cases between the onset and the taking of the specimens

That is a thing that one can avoid only by having more knowledge of what we may expect to have to do So I think it is very difficult to draw some firm conclusions on some of these studies, and I would say very strongly that in all surveillances fecal specimens must be taken and not just fecal swabs Proper fecal samples should be taken immediately as the individual is observed

This points to one of the very great problems the difficulty of sorting things out

One other small point I would like to mention is that I wondered if you Dr Cox thought there was some evidence of interference For example, in Chart No 6,* the child is fed Type 2 vaccine and there is no antibody response to Type 2, or whether it was just a vaccination break, or whether that child may have had Type 1 virus when you fed Type 2 vaccine

As the final point I would be very interested to obtain any further information on Coxsackie 4 which anybody else has This point is interesting

DR. COX This is the case that occurred in Medellín, am I correct? Of course no one can be absolutely sure as to what happened here We know that some of the early batches of vaccine that were sent to Medellín were low in virus titer Medellín and Managua were the first places to which we shipped liquid vaccine We thought we were sending vaccine with a virus titer of $10^{7.5}$ to $10^{7.8}$, but found after it had been shipped, much to our dismay, that the virus titer had dropped appreciably, even though the vaccine had been stored at minus 20° So, actually, the vaccine that went to Managua had a titer of about $10^{4.5}$ and the vaccine that went to Medellín had a titer of about $10^{4.8}$

* See p 674

The Medellín case could conceivably be considered a vaccine break, just as Dr. Ramos Alvarez dismissed the first three vaccinees in Mexico as vaccine breaks All I can say is that we have honestly done our best to present all the data that we have today, on approximately two million doses of our product.

We fully realize, as you have brought up, that one should be located right on the spot to collect complete information But keep in mind that this work was done in the field, under the joint auspices of the Colombian Government and the Pan American Sanitary Bureau, and that our laboratory is about 4,000 miles away. Frankly, some of the specimens supposedly shipped to us never reached our laboratory But these same problems arise, whatever the product concerned, with any surveillance program undertaken on such a large scale

We frankly believe our vaccine strains to be some of the most attenuated viruses that we have ever had experience with However, if anyone here makes the statement that it is possible to go into the field on such a large scale with a biological product and have absolutely no follow-up problems at all, then that individual undoubtedly shows his ignorance of biological products as well as of what can happen under general field conditions

CHAIRMAN ARMSTRONG Dr Sabin

DR SABIN. I think my old friend, Dr Cox, is overly-sensitive, and he has jumped to the defensive before he has even been attacked

I do not know what other people might have been shaking their heads about, but if he caught me shaking my head during the course of the presentation, I assure you it had nothing to do with the things that he said

Then I would also like to remind my good old friend, Dr Herald Cox, that it is not a good defensive to attack something else Therefore I hope that I may be permitted to make a few statements, both about the presentation of Dr Roca Garcia, and particularly what Dr. Cox had to say

First of all, I think this is an excellent and very fine study, and it is most reassuring The problem presented in the last two cases is a difficult one to decide whether or not the Coxsackie 4 is responsible It may very well have

been, but to me the most enlightening evidence is the fact that the Type 3 virus recovered from those two patients in tests in monkeys—and I do not care whether the needle went in exactly in this particular case—was not virulent virus.

You see, you may get a slight difference where you inject it intracerebrally. If you had a really virulent virus at the time, such as we used to work with in the intracerebral inoculation in the frontal lobe or any other way, it would have shown up.

Now, what some may have been shaking their heads about is what Dr. Cox himself said: It is a pity that the sample was gotten so late, so that you cannot have a better identification

I think the other studies, the other cases analyzed, show what happens when you go into an area where polioviruses are already spreading

Now, he said that no such analysis had been presented before, in the material that Dr Skovranek presented—I am sure that Dr Cox has been busy every night, and he has not had a chance to read the full documentation that is there The full documentation contains the one instance that had to be investigated which showed clearly that the type of virus fed was not involved with the incidence of transitory weakness that occurred

The full documentation of the material presented by Dr Smorodintsev and Dr Chumakov contains precisely the same kind of analysis I spent ten days going over all of that material, not only in summarized form, but in looking at the original material and the statement can be backed up by data that are presented

So I would say, first of all, that Dr. Cox should not be so sensitive and he should not try to defend himself against a charge that has not yet been made, and also, I hope that he will not try to defend himself by attacking something else

I should say a word about the other field study If Dr Ramos Alvarez had presented his data fully, he would have showed that there were periods during wh ch there was no great incidence of poliomyelitis in 64 000-some-odd persons and there were no cases to investigate The cases in the midst of the epidemic of Type 1 poliovirus in Mexico City, as he showed were occurring some in those vaccinated and some in the unvaccinated

There is ample evidence to indicate that some people in such an environment do not become immunized; he drew the only possible conclusion, which was a valid one, that there are some cases that, despite the fact they obtained Type 1 vaccine, nevertheless developed Type 1 paralytic polio later.

I hope that this straightens out the record

If there are any other questions that Dr. Cox would like to have answered, I would hope for an opportunity to put things straight.

Dr. Abad Gomez I am going to speak again, not as an epidemiologist or a virologist, by any means, but just as a health officer of Colombia

We have been, as much as we can, most careful in looking for cases that might occur We have not had a large staff to assign immediately to suspect cases and collect everything But, as I said, we have 13 health centers, and most of the families in Antioquia are aware of the fact that they can go and take their children to the hospital or health centers if they are ill

I presented the facts to show that cases of polio are much less than we expected to have up to this time, after the vaccination I even told Dr Paul yesterday that he might have two cases in which attenuated Type 3 virus could be involved perhaps I am very glad to learn that one recovered very well, and another one, in whom Coxsackie A was isolated, was also a very mild one

As a health officer, I am therefore happy to state that we had much fewer cases of poliomyelitis than we expected for the season

I would emphasize the fact that in my country where the Salk vaccine cannot be given to everybody because we just do not have the money for it we did well vaccinating with the attenuated virus Even after analyzing these cases, and doubting that they can be incriminated at this time I would go ahead and vaccinate my whole state if I had the trivalent vaccine, because I know for sure, that this is much better than the wild virus that is going on there all the time, without any defense whatsoever

Dr Lebrun I should like to add a few words to this discussion and present my own philosophy on the matter

If no one here has brought irrefutable proof that live vaccine in nature is incapable of revert-

ing to the virulent state, it seems to me that no one has presented irrefutable proof either that the vaccine is capable of doing so under the same natural conditions

All field trials have shown not only that there is sero-conversion, which allows us to assume that there is adequate protection of the population, but also an incomparably lower incidence of the disease in vaccinated groups than in the non vaccinated This incidence is so very low in these large groups that it is perhaps due to the few individuals who do not become immunized, for one reason or another, such as interference with another circulating virus, faulty technique in vaccine administration, or absence of virus fixation in the cells of the intestinal tract

Thus, for example, in the military camp in Leopoldville, where we vaccinated in spite of a current diarrhea epidemic, the rate of sero-conversions was 47 per cent that is, the lowest in the entire city.

Moreover, I personally believe that the circumstances surrounding the Leopoldville study are interesting The objection could be raised that the epidemic that broke out three months after the vaccination was the result of increased virulence in the virus strain through successive passages If that were so, one would have to explain why the epidemic did not develop in the 40 per cent non immune of the community where the virus was introduced on a mass scale and deliberately, whereas it developed just where it could not have been brought in except through carriers.

We should note in passing that during the 18 months elapsed there appears to have occurred, in many of the countries studied here, an increase in virulence of the wild strain *In the* past few months an increase in polio cases has been observed even in the United States, a country that was extensively vaccinated with killed vaccine and where the medical profession's interest in polio is not new

Injections into monkeys are no doubt impressive and, as Dr. Abad Gómez has said, during the first three days of this Conference sanitarians could well ask themselves with concern what they had accomplished. But it seems to me that, chiefly, the various authors were not altogether agreed on the value of the different techniques employed May I stress, moreover, the highly artificial nature of these trials in relation to what happens in nature: intraspinal or intra cerebral injections, at times in very high doses with local trauma, on the one hand, and oral *administration of moderate doses on the other* And under these very severe conditions we still do not obtain more than a relatively weak rate of paresis or visible paralysis

In the final analysis, what the sanitarian is trying to prevent is, first, the clinical lesion, and then the microscopic lesion, if that is possible

In conclusion, from all that I have heard here, I can find no completely convincing argument to induce me to refuse to the populations with whose health care I am entrusted the benefits of this new weapon to help alleviate, under existing conditions, the human and social problems raised by polio in a country of the social, economic, and sanitary structure of the Congo. In fact, I do not believe that I have the right to wait To wait for what? For how long?

The Cutter incidents have been cited as a justification for great—perhaps too great—caution In fact, nearly 30 years after the Lubeck incident there are still those who are not convinced of the value or of the innocuity of BCG

To wait for what? Countries with very high health standards are reluctant to make large-scale trials and, of course one could always criticize the inadequacy of figures On the other hand, in the other countries there are so many diverse factors—political, economic, sanitary, religious, or philosophic—that can interfere and that have in fact affected the campaign, that there too it will always be possible to criticize this or that point, or to reject a given experiment

We must, without a doubt, be cautious But I do not believe that excessive caution should sterilize our work, and we must not forget that every day that passes brings new cases of illness in our world It is our role to weigh carefully the existing risks, and then to decide what appears to us to be the most judicious course

CHAIRMAN ARMSTRONG Dr Roca-García.

DR ROCA-GARCÍA: I just want to make a comment on the question that Dr. Dick raised about the stools being collected quite late in those cases. Fortunately, in the two cases where

Coxsackie virus infection was involved, one sample was taken promptly I do not know if Dr Dick has seen Charts No 6 and 7,* but as we can see, one patient developed paralysis on 1 February and the stool sample was collected the next day We have evidence that the other patient had a Coxsackie infection the day she arrived in the hospital, for Coxsackie antibodies were demonstrated in her blood sample that same day So, in this case, we have an alibi

CHAIRMAN ARMSTRONG. It would have been nice to have had an earlier sample, though

DR ROCA GARCIA: Yes, most of the samples were obtained late.

CHAIRMAN ARMSTRONG. Dr Bodian

DR BODIAN. I am afraid the hour is late, but as I told the Secretariat on Monday, it will not be possible for me to be present this afternoon, and I would like to make a few remarks if I may, which I otherwise would have made in the afternoon

It is my own loss that I cannot be present because I have enjoyed every bit of this Conference

Ever since 1939, when Dr Howe and I began to feed poliovirus to chimpanzees and to study their clinical, pathological and immune responses, I have had a great interest in all problems of poliomyelitis and poliomyelitis immunization, including the use of live virus as well as killed virus vaccines I consider myself as non partisan in relation to the two types of vaccines, and I am hopeful that live virus vaccine may find an important place in public health practice Partisanship would only reduce the effectiveness of a most important ingredient of progress in public health as far as that is based upon scientific advance. This ingredient is critical analysis, especially critical analysis of one's own results, and the invaluable criticism of those who do not have a personal stake in a specific idea or product I can assure Dr Abad Gómez that critical analysis in every stage of development of inactivated poliovirus vaccine was infinitely more important than courage and faith, although these have their place also

As a consultant to the Surgeon-General of the U S Public Health Service and to The National Foundation and as a member of this important international meeting, I should like to conclude my discussions by referring to three important questions which have been only barely touched upon in this Conference, partly because of the absence of information and partly for other reasons Because of this, these questions may not receive sufficient attention this afternoon when the Conference will attempt to summarize the large numbers of extremely important contributions presented this week Great progress has been made in the past year or two and the contributors are to be congratulated upon their achievements Those who feel that some of us are too slow to accept their conclusions should remember that organizations responsible for the regulation of vaccines which may be distributed internationally in commerce face special administrative problems which do not confront countries which procure or produce vaccine which will only be used locally Two of the questions which I shall raise deal with this special responsibility which confronts some of us who may seem to be timid and without faith

The three problems which I believe have not received adequate attention at this Conference are

(1) The duration excretion and extent of community national and international spread of poliovirus of vaccine origin This question is related to the problem of the degree of continued stability of the property of low virulence under environmental circumstances which may be related to the initiation of epidemics.

(2) The control of safety and potency of successive lots of vaccine viruses This problem is related to the question of whether field trials which indicate the safety of one investigator's viruses are to be considered as also having demonstrated the safety of all viruses now being employed And what about successive batches of any one virus? Should each one be used on a small scale before being used in mass trials? What criteria are to be used in the laboratory and in the field for ensuring *continued* safety?

(3) The problem of how to make a critical assessment of the origin of poliomyelitis cases appearing in vaccinees and their contacts after the inception of vaccine feeding programs

All three of these questions raise, in addition,

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* See pp 674-675

issues of perplexing difficulty in relation to the collaboration of laboratory scientists, epidemiologists, public health officials, manufacturers, and government administrators. Are their responsibilities mutual or separate? Should public health officials share responsibility for basic scientific work upon which their decisions are based, and should laboratory scientists share responsibility for the conduct of field trials? Should the individuals who offer virus strains for use in field trials play an active role in the testing of these viruses in the field in advising about the conduct of the trial, in conducting the laboratory work involved in the study and in the diagnosis of suspected cases of poliomyelitis?

What would your opinion and mine have been if Dr Salk, instead of Dr Francis, had conducted the field trial of a new product tested the specimens, diagnosed suspect cases of poliomyelitis and interpreted the results? This is not a question of honesty or competency It has always been considered a question of propriety and of eliminating the confusing issue of partisanship, which is a danger in every scientific investigation, as it is an ingredient of all human activity

I realize that these questions will not be answered easily, but I believe that they are as important as many of the problems which we have discussed in detail in the Conference

There will always be a period of doubt before each step in an advancing development of a new product The importance of this Conference is that it allows each one present to decide how much doubt exists and how to discharge his own responsibilities

Dr Abad Gomez Just a very short remark I like the approach of Dr Sabin about agreement, and I think we are coming to an agreement I agree with Dr Bodian about very many of the things he said

I now have a very small different approach than yesterday Yes it is not only courage and faith, there will have to be wisdom

I was conferring with Dr Henderson this morning We were talking about whether the monovalent was better than the trivalent vaccine, and now I am for the trivalent type of approach That is: wisdom, courage, faith, and few drops of skepticism, to make the cocktail better.

Dr Soper: Mr Chairman, in the light of Dr Bodian's remarks, I would like to state that, although the work in which the Pan American Sanitary Bureau has been engaged has been carried out in connection with the laboratories and the facilities of the Lederle Company, there has been no partisanship on the part of the Organization

The fact that the other viruses were not used by the Bureau was due to circumstances beyond the control of the Director

The limitation of the work has not been due to the Bureau's reluctance to have additional participation

It should be a matter of record that before the Pan American Sanitary Bureau undertook any work outside of the United States with attenuated live poliovirus vaccine an approach was made to the Surgeon General of the United States Public Health Service to establish its own criterion for participation in the planning of programs, and an invitation was extended to participate even in the collection and examination of such materials as might be indicated from the field

I want to disclaim all partisanship for the Bureau My willingness to work with the two sets of viruses was due to an essential agreement in the information received independently from the two producing sources

There is another point to be emphasized at this meeting, and that is that we are not, here, interested only in numbers There is a statistical value to increasing numbers but all of our statistical information, all our quantitative information, disappears on the basis of any qualitative change, and the numbers that accumulate will not prevent that qualitative change, if and when it occurs

And so I rather disparage some of the remarks this week regarding the importance of the numbers of people who have been vaccinated

The experience with the 17-D yellow fever vaccine, an attenuated live virus vaccine, merits a brief consideration The vaccine was first field tested in Brazil in 1934 Some 40,000 people were given the vaccine the first year, and rather careful field controls were carried out There was no evidence of any difficulty, and in the fol-

lowing year just over a million people were vaccinated with no recorded difficulty

In the third year, however, some fatal cases of yellow fever did occur among people who had been vaccinated several months previously and should have been immune The field investigation suggested there had been partial loss of antigenicity in some of the strains of vaccine used, in the course of multiple subcultures A return to earlier subcultures, with the introduction of the seed-lot method of preparation of vaccine, solved this problem.

The following year, after 1,600,000 people had been vaccinated it was discovered that certain lots of vaccine were producing post vaccination hepatitis The elimination of human serum as a diluent in the manufacture of vaccine solved this difficulty

The following year a few scattered cases of encephalitis were found in the vaccinated areas, and studies eventually resulted in the elimination of certain sub-strains of vaccine virus which had an apparently increased neurotropism

After encountering and solving the three difficulties cited, the live virus yellow fever vaccine produced in Brazil has given no further difficulties during the past 17 years. But this does not mean that continued observation is unnecessary. We are not interested, I believe only in knowing how many millions of people have been vaccinated with attenuated live poliovirus vaccine, but we are very keenly interested in any information that suggests qualitative changes, when the vaccine is used in the field

CHAIRMAN ARMSTRONG The Committee on Summaries is not ready to report yet, but the report will be completed soon. In the meantime, if any one has problems which should come before the meeting and which have not been adequately explored, would they please present them at this time

DR SMADEL While we are waiting for the arrival of the report, I would like to make a few remarks

I, too, want to congratulate the participants in this Conference and those who have contributed to its success by the reports of their laboratory work and field work I would like to talk particularly about those who have done the field work It seems to me that in science we sometimes fail to acknowledge adequately episodes which are really heroic. Many of the field studies we have heard reported this week belonged on the heroic side, and most of them were courageous

One of the things that has impressed me, despite the fact that all of the information is not yet in, is that we can be pretty assured of the high degree of safety of all of the various strains that have been used, when administered to the individual vaccinee. However, we are not yet, and I suspect we will not be for a long time, in a position to assign an absolute value for the safety on any of the strains

While we can speak with some confidence of safety for contacts of vaccinees I think we are on more tenuous ground here In part this is because we have not had the opportunity to make enough observations over a period of time Studies which will provide such information are planned and must be carried through

There is one aspect of the live attenuated vaccine that we have almost neglected in the discussions this week, and I think it deserves more consideration. This subject has to do with the procedures employed for the commercial production of live polio vaccine The data presented by Dr Cox in his first paper dealing with observations on sequential lots of vaccine prepared under his direction provided the only information of this particular kind during those sessions which I attended I think it is clear that all of the field work that has been reported this week was done with a relatively small number of lots of vaccine, which were produced on a laboratory scale or, at the most, on a pilot plant scale

Much work remains to be done by the biological houses in order to translate the experience gained from small scale production of a relatively few lots of vaccine, made under very careful conditions, to the development of standard commercial production methods which can be done by rote You who have used the live vaccines in the field may think me unnecessarily conservative when I emphasize the need for an adequate experience in commercial production of the new vaccine However, I shall only remind you that undue haste in progressing from small to large-scale production of killed polio vaccine was, in part at least, responsible for the Cutter incident Now that the problems con-

nected with killed polio vaccine have been solved and the Salk type preparation has come into wide use in the United States, we here can afford the time required to iron out all the problems connected with commercial production of live vaccine

There are some regulations connected with *biological products* which I think should be *brought to your attention* It is *not possible to* export a commercial product from the United States of *America* unless it is licensed for use within the United States In order for a product to be licensed for use within the United States *it must have been shown by a biological, commercial producer that the material is useful, that it is safe, that it is potent and that it can* be consistently produced.

We have heard comments during the week of the desirability of licensing a live polio vaccine The United States Government, through its various agencies—the *Department of Health, Education, and Welfare in turn the Public* Health Service, in turn the National Institutes of *Health, and in turn the Division of Biologics* Standards—issues a license for a biological product only after a specific request for the issuance of such a license has been made by a commercial house which has *demonstrated* its capacity to produce a satisfactory material Thus, the procedures concerned with licensing in this country may be different from those in force elsewhere

CHAIRMAN ARMSTRONG Dr Lebrun

DR LEBRUN *(through an interpreter)* I understand full well, and I believe that everyone here is fully aware of the reasons for the prudence of the United States with respect to the live virus, after the regrettable incidents of the last few years. *Everyone is fully aware* of this, and everyone understands very well what the previous speaker has just said: that in this country they have gained the right to wait, and above all, they have the possibilities to wait Nevertheless, I would like to draw the attention of the Conference *to the fact that three quarters* of the world's population live in under-developed areas Those areas have no *time to wait*, they have no possibilities to wait And we must *not* forget that the conclusions *derived* from our Conference may be used by certain timorous administrators *in other countries to try to prevent* the benefits of this new vaccine from being extended to other populations We must, *in my opinion, beware of introducing into the conclusions of this Conference statements which, under the guise of excessive prudence, would exercise* an impact upon the commercial aspect of the matter and might hinder the exporting to countries which are not capable of producing themselves a vaccine that may prove useful for other populations

DR MURRAY The remarks that Dr. Smadel made apply immediately to material that is produced in the United States

I would like to say that, although the information which has been presented at this Conference represents a very valuable contribution in enlarging our knowledge about these agents, it does not cover the entire picture, as far as making available a safe and potent product There are other things which come into consideration

We did mention briefly such ancillary factors *of safety as the presence of wild viruses, B virus*, and so forth To our mind these aspects have not been fully settled yet

Then there is the question of the strains to be used in the vaccine

Obviously, I think one has to admit that the studies, the results which have been presented, apply only to the strains, the strain pools, the particular lots of vaccine which have been under study and the data presented to this Conference

What is going to happen when new batches are prepared? What are the criteria for assurance of safety and potency? What criteria will we find ourselves able to use in judging "improved" strains—and I put that word "improved" in quotation marks Does that mean that every new improvement that comes along must, in itself, be subjected to extensive field trials, in order to validate it?

The opportunities for field trials of this sort are diminishing as time goes on, in the same way that the opportunities for carrying out clinical work with the killed vaccine have diminished in the United States. At the present time it is almost impossible to find an investigative situation where the potency of the vaccine may be studied clinically.

One of the major problems that must be resolved, before licensing or approving a live

poliovirus vaccine, is the establishment of adequate laboratory controls for safety.

At the present time various laboratories are reporting divergent findings regarding monkey neurovirulence, which is one of the factors used in selecting these strains; and the relationship of such laboratory findings to potential pathogenicity for human beings, after oral administration, is not clear But these things certainly must be resolved before these products can be generally accepted for wide distribution and manufacture

I think those are a few of the general remarks I wished to make Dr Bodian presented some this morning, and I do not wish to repeat those.

Dr Courtois This is a question of field trials, on the one hand, and of control, safety, and effectiveness, on the other The exchanges of views we have had in the past few days have convinced all of us that today, when it is a question of laboratory tests, because the tests have such a fragile base we had no absolute criterion, and no over-all criteria, to allow us to reach a conclusion or to compare the various polio vaccines used, as regards both their efficacy and their innocuity for man For no other vaccine whether live or not were there so many and so severe tests on animals but for this virus which in nature seems to be interested only in man, there is always an unknown factor. which makes it impossible to transpose directly to man the results obtained in animals no matter how close the animal is to man on the scale of evolution Only with vaccination campaigns carried out with the best control possible and only by continuously improving our methods, will we be able—through the results obtained in the years to come—to reach a valid opinion on the situation

The problem of the safety and efficacy of the vaccine as applied to man in the long run will be an epidemiological problem in which laboratory tests must take their rightful place, which is not secondary

Moreover let us suppose that by an extraordinary circumstance—and I emphasize that this has by no means been proved—suppose that after numerous passages these vaccines should become more virulent Is there a real reason to adopt the attitude of terror which the first reports wanted us to accept? I do not think so The longitudinal studies done by Gear, Gelfand, Fox, and many others, show us that, in the worst hypothesis—not demonstrated before—we would have, after successfully immunizing millions of individuals, introduced yet another virus among all the polioviruses which these investigations have found circulating on all continents

As for the question of legitimacy and efficacy of live vaccines in times of epidemics, we believe that most tropical countries are chronically threatened with more or less important outbreaks of polio epidemics What can we do under present circumstances?

We do not think that the use of killed vaccine is indicated in that case, for many reasons, not only economic reasons, but also because of the undeniable effect of the injection itself As Dr Sabin states, in connection with interference what we must do is to oppose, not 10 against 10 000, but 10 000 against 10 000 Victory will always come to the largest battalion This is what we were the first to realize and publish during four epidemics that we had when we successfully used the CHAT strain

Perhaps in the laboratory one can ask the questions What might have happened if we had not interfered, and if we had not used the live vaccine? Would the epidemic have stopped by itself? Assuming this, we can also suppose that there would have been double the number of paralytic cases But in cases dealing with human lives and where these can benefit from a doubt we must let them profit from that doubt rather than satisfy any scientific curiosity that we might have, no matter how legitimate it may be

Chairman Armstrong Dr Gard.

Dr Gard There is one subject that has been brought up during the course of this Conference on several occasions a very important subject that, in my opinion, has not been discussed as thoroughly as it deserves

It is important because it deals with a situation where the courage that Dr. Abad Gómez was talking about it is needed; and where the further development in the vaccine field may be affected very much by the decisions taken.

nected with killed polio vaccine have been solved and the Salk type preparation has come into wide use in the United States, we here can afford the time required to iron out all the problems connected with commercial production of live vaccine

There are some regulations connected with biological products which I think should be brought to your attention It is not possible to export a commercial product from the United States of America unless it is licensed for use within the United States In order for a product to be licensed for use within the United States, it must have been shown by a biological, commercial producer that the material is useful that it is safe, that it is potent, and that it can be consistently produced.

We have heard comments during the week of the desirability of licensing a live polio vaccine. The United States Government, through its various agencies—the Department of Health, Education, and Welfare, in turn the Public Health Service, in turn the National Institutes of Health, and in turn the Division of Biologics Standards—issues a license for a biological product only after a specific request for the issuance of such a license has been made by a commercial house which has *demonstrated* its capacity to produce a satisfactory material Thus, the procedures concerned with licensing in this country may be different from those in force elsewhere

Chairman Armstrong. Dr Lebrun

Dr Lebrun *(through an interpreter)* I understand full well, and I believe that everyone here is fully aware of the reasons for the prudence of the United States with respect to the live virus, after the regrettable incidents of the last few years Everyone is fully aware of this, and everyone understands very well what the previous speaker has just said that in this country they have gained the right to wait, and above all, they have the possibilities to wait Nevertheless, I would like to draw the attention of the Conference to the fact that three quarters of the world's population live in under-developed areas. Those areas have no time to wait, they have no possibilities to wait And we must not forget that the conclusions derived from our Conference may be used by certain timorous administrators in other countries to try to prevent the benefits of this new vaccine from being extended to other populations. We must, in my opinion, beware of introducing into the conclusions of this Conference statements which, under the guise of excessive prudence, would exercise an impact upon the commercial aspect of the matter and might hinder the exporting to countries which are not capable of producing themselves a vaccine that may prove useful for other populations

Dr Murray The remarks that Dr Smadel made apply immediately to material that is produced in the United States

I would like to say that, although the information which has been presented at this Conference represents a very valuable contribution in enlarging our knowledge about these agents, it does not cover the entire picture, as far as making available a safe and potent product There are other things which come into consideration.

We did mention briefly such ancillary factors of safety as the presence of wild viruses, B virus, and so forth To our mind these aspects have not been fully settled yet

Then there is the question of the strains to be used in the vaccine

Obviously, I think, one has to admit that the studies, the results which have been presented, apply only to the strains, the strain pools, the particular lots of vaccine which have been under study and the data presented to this Conference

What is going to happen when new batches are prepared? What are the criteria for assurance of safety and potency? What criteria will we find ourselves able to use in judging "improved" strains—and I put that word "improved" in quotation marks Does that mean that every new improvement that comes along must, in itself, be subjected to extensive field trials, in order to validate it?

The opportunities for field trials of this sort are diminishing as time goes on, in the same way that the opportunities for carrying out clinical work with the killed vaccine have diminished in the United States. At the present time it is almost impossible to find an investigative situation where the potency of the vaccine may be studied clinically

One of the major problems that must be resolved, before licensing or approving a live

demic and pre-vaccination immunological surveys, one could expect that of the vaccinated children, about two-thirds had originally been fully susceptible to Type 1, whereas in those that remained unvaccinated at the time of reporting only one-third belonged in the category of originally susceptible infants

Therefore, attention to this difference in the composition of the populations will tend to increase the number of expected cases in the vaccinated group, and also the significance of the figures

CHAIRMAN ARMSTRONG Dr Fox

DR. FOX There were two points made during the course of this meeting, one earlier by Dr Dick, and one somewhat later by Dr Langmuir, that seemed to me to require a bit of comment An impression that Dr Dick created early in the meeting when he gave his very interesting lead-off paper, was one of considerable apprehension as to what sort of an unpredictable juggernaut we might have let loose on the population by turning loose an attenuated (at the time) virus Also, if I remember rightly, he compared the prevention of 10 current cases with the creation of 100 cases at some future date

This, of course hinges directly on the problem of strain stability, which has been discussed very amply, and about which much more information is needed But the question which concerns me is that I have the feeling that we are overly concerned, or at letast some of us are overly concerned, about this particular problem

If one looks at most parts of the world, the chance of any given individual acquiring an infection of poliovirus at some time in his life is with a particular type of virus, let us say, on the order of 80 to 100 per cent, and there is a good deal of evidence, I think, to show that the earlier he acquires this infection, the better it is for him

At one stage I was reckless enough to say that if we did not have these strains available, I would feel that I was conferring a favor on an individual by grabbing the first wild strain I could get my hands on and feeding it to that individual in the first year or two of life, rather than letting him wait until nature saw fit to infect him

I believe this kind of thinking has its application to the utilization of these strains that are far from wild strains By and large, if you do a thorough job of immunization, you will have pretty well removed this individual from further danger, and if you have done a reasonably thorough job on the population, you will not have very many susceptibles left in the population for this strain to migrate to

As a matter of fact, we do not have too much evidence that these strains are able to persist indefinitely in the population

The other point in my mind was raised by Dr Langmuir when he was talking about the problem of surveillance and he used the term "a fine-toothed comb" I do not mean to imply that careful surveillance is not a good thing I would be quite interested in the end in knowing the general range of reaction production that a particular vaccine or type of vaccine can give rise to

But the question really at issue is the question of paralytic disease He suggested that we might be using the results from Costa Rica—I believe that was under discussion at the time—to guide us in our operation of the strains in this country If we were entirely dependent upon the results in Costa Rica and some of the other Latin American countries where, because of the age range of the susceptible population, the tests are really only in very young age groups, I would say that his point had much more validity with respect to translation to his country

But we are not so dependent We have, increasingly, a very great amount of information about the effect of strains on one type or another in population groups that are quite comparable to our own in terms of the proportion of non-immunes in the more advanced age groups in which susceptibility to the paralytic effects of infection is presumably greater

It seems to me that, particularly in that category of test, the thing that we are still most interested in is to be sure that the vaccine does not produce serious paralytic or other damaging types of disease

Suppose it gives three days of fever occasionally, or maybe even a stiff neck once in a while, This would not worry me too much, although, I confess I would rather it did not do anything but just pass harmlessly through the gut and immunize

and these in turn must rest upon the evaluation of the observations.

I am referring now to those field trials in which the investigators encountered an epidemic of polio

It was more or less stated, during the brief discussion following some of these communications, that you simply cannot evaluate such trials

I cannot help being of a slightly different opinion I think we can evaluate the results We have to be extremely careful in doing so, but I think there are methods, and they should certainly be applied

If I may again use the blackboard, I will try to explain how I look upon this problem and suggest some methods by which we can arrive at *an opinion upon which we can base decisions of* what to do in such a situation

May I use the blackboard for a moment?

CHAIRMAN ARMSTRONG Surely

DR GARD *(at the blackboard)* Let us assume that we have a population in which vaccination is performed and we can record the rate of vaccination from day to day and from week to week

I should perhaps say at this point that any evaluation of observations of this nature depends entirely upon the accuracy of the primary data available, that is, the thoroughness of the knowledge of what is being done, and what is happening

But, suppose we have a situation where such accurate data are available and in the course of the vaccination campaign an epidemic appears

In order to meet the objections raised by Dr Hammon during the discussion we will have to divide the epidemic in a series of brief periods and consider each period separately Only by so doing can we correct our calculations for the intensity of the epidemic at each particular moment

The best procedure should be to calculate what proportion of the cases occurring in a particular period could be expected to occur in the vaccinated if the distribution was determined by chance alone. We then arrive at a series of expected numbers, and can put those against a series of observed numbers of cases. A summation of expected and observed numbers of cases should be legitimate, and consequently also a statistical evaluation of the classical type.

You have, of course, to be extremely careful to remove all bias in favor of the vaccine. Thus, you have to try to estimate when a reported case of poliomyelitis was actually infected, because it is the immunization status of the population at the moment of infection that is of importance That is, you have to antedate your polio cases.

In going over the primary data of the epidemic that were made available to me by Dr Plotkin, I tried—as an illustration of the principle—to arrive at an estimation of the situation in the Belgian Congo, and I may just summarize that by a few figures

The procedure was to antedate the polio cases two weeks and calculate week by week the expected number of cases in the population reported vaccinated at the beginning of the week, and then sum up these estimated numbers For the different areas recorded by Dr Plotkin, I arrived at the following figures 7 52 cases expected, 2 95, 1 87, 0 70, 0 89, 1.92, and 1 92

Now these figures are generally too small to serve as a basis for statistical calculations and we have to group them together somehow

In order to have expected numbers of at least 5 we have to form three groups of 7 52, 5 52 and 4 73, respectively The actually observed cases were four three, and two

I have then taken the liberty to exclude one case that quite obviously must have acquired infection before vaccination—a case where symptoms were present two days after administration of the vaccine The total number of cases in vaccinated children would then be 9, as against 17 77 expected

This gives us a X(chi) square of 4 328, with one degree of freedom I am sorry I cannot remember offhand the significance levels It does not carry a very high significance, but as far as I can see, the degree of significance is sufficient to permit a statement that there was a probable protective effect of the vaccine in this population

There is one additional factor here which has to be taken into account, that is, the question of the comparability of the vaccinated and non-vaccinated populations.

As pointed out by Dr. Plotkin, there was an over-representation of young children among those vaccinated and, on the basis of pre-epi-

demic and pre-vaccination immunological surveys, one could expect that of the vaccinated children, about two-thirds had originally been fully susceptible to Type I, whereas in those that remained unvaccinated at the time of reporting, only one third belonged in the category of originally susceptible infants

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Properties of Polioviruses Excreted by Orally Vaccinated Persons

Several investigators compared the properties of the vaccine virus with the virus subsequently excreted. As low monkey neurovirulence, that is to say, an absence of effect after intracerebral inoculation and a low degree of activity by intraspinal inoculation, was the criterion for selecting the strains in the vaccines, this property was regarded by many as the most important measurable character as to whether or not the virus changed in the course of multiplication in the human alimentary tract. Evidence that there was reversion towards increased monkey neurovirulence in the virus excreted after vaccination was obtained by several workers in different countries. However, in the studies reported there was no evidence of progressive increase in neurovirulence for monkeys during prolonged multiplication in the vaccinated individual or after serial passage of virus in human beings.

As already mentioned, encouraging results were reported with certain recently described genetic markers of polioviruses. These markers could be used for detecting altered strains with greater neurovirulence than that of the vaccine virus itself. More work is needed on this subject because of the importance of being able to differentiate excreted vaccine strains from "wild" strains of poliovirus.

Field Studies

Design

More than twenty field studies were reported from no less than fifteen countries. Some have been organized in populations already partly vaccinated with the inactivated Salk type vaccine. Most, however, have been done in highly susceptible populations which had not been given inactivated vaccine.

Both small- and large-scale studies have usually involved the sequential feeding of children, of 0 to 12 years of age or less, with the three types of attenuated poliovirus, but in some of the most recent studies the simultaneous administration of all three types has been done with apparent demonstration of immunization to all three types. Following the oral vaccination, there has usually been a period of surveillance lasting several months or longer, in which both vaccinated persons and their associates have been observed rather closely. In the larger studies, serological investigations have been done on selected samples of the population, and the surveillance for illnesses after vaccination has taken different forms based somewhat on existing local public health procedures for detecting illness of any kind in the community at large. Thus, in some of the studies where a large segment of the population has received the vaccine, attempts to estimate the paralytic poliomyelitis rate in the vaccinated as compared with the unvaccinated group have been made. This is admittedly a procedure difficult to interpret, particularly from a statistical standpoint.

Antibody response

The studies designed to test the efficiency of the vaccine have yielded different results in different environments. For instance, on the basis of the antibody response of vaccinated children who lacked antibodies before vaccination, in some studies it was reported as being only 50 to 60 per cent effective, whereas in others it was about 90 to 100 per cent.

The antibody response depends on the successful establishment of alimentary infection and evidence was produced to indicate that agents which interfere with this alimentary infection will limit the development of immunity.

The response of new borns and children of varying ages and adults, including pregnant women, have been studied and, in general, infants and children responded better than adults. The immune status of the individual prior to feeding obviously had an important bearing on this response. The dose of the vaccine also influenced the development of immunity.

Interference

The role of interference received much attention. Some viruses found in the alimentary tract, as well as other polioviruses may prevent the infection with attenuated vaccine virus from becoming established. It was clear that this is a problem which demands much more study.

Data are lacking regarding the role of other enterovirus infections in possibly enhancing the invasiveness of attenuated polioviruses.

Earlier reports had suggested that if all three types of polioviruses were administered simultaneously, there was interference between them. However, recent studies have indicatd that when

SUMMARY OF THE CONFERENCE

CHAIRMAN ARMSTRONG: The Summary Committee has brought forth its efforts and labors, and the report it prepared will be presented by Dr. Payne

DR. A. M-M PAYNE *(Chief Medical Officer, Endemo-Epidemic Diseases, World Health Organization)* The document which is before you has been prepared by an anonymous group, anonymous because they have attempted to summarize the opinions of all participants at the Conference and to give an objective reflection of the different views expressed here No attempt has been made to draw conclusions, or to make recommendations since that was not the purpose of this Conference which was essentially to bring all available data into the open onto the table and to elicit the differences of opinion which may exist, in the hope of eventual resolution by further work

After Dr Payne read the document, it was reviewed and discussed by the participants and the following text was unanimously approved.

The value of the inactivated (Salk) vaccine in the prevention of paralytic poliomyelitis is well recognized by this Conference and its continued widespread use is strongly recommended However, there is general appreciation that there are limitations to its use in many parts of the world and that it does not prevent alimentary dissemination of wild polioviruses, which constitutes a hazard to non vaccinated persons There is, therefore, a place for a vaccine that may be of lower cost, simpler to use, and potentially capable of having a greater protective effect on a community basis The use of a vaccine that can be administered orally has advantages Further, there is the possible advantage that such a vaccine virus might replace wild viruses in nature, thus eliminating the threat of paralytogenic strains.

It is recognized, however, that the use of a product that spreads beyond those originally vaccinated represents a radical departure from present practices in human preventive medicine.

The very difficult problems in the development, control, and evaluation of the safety and effectiveness of experimental live attenuated poliovirus vaccines were the main concerns of the Conference The solution to many of these problems remains to be found.

Properties of Attenuated Polioviruses Used as Oral Vaccines

There is general agreement that the vaccine strains reported upon at the meeting were of low virulence for primates Repeated reference was made to the recommendations of the WHO Expert Committee which met in 1957 It was apparent, however, that the results being obtained on inoculation of monkeys by different investigators varied considerably—a circumstance attributed by some discussants to differences in technique and, perhaps, animal susceptibility. The desirability of standardization of the techniques was emphasized

Thus far the criteria of attenuation have depended upon the reduction of neurovirulence for monkeys, and, indeed the strains under study have been selected on this basis. However several participants expressed the need for study of properties other than neurovirulence which might be used as additional evidence for the safety of vaccine strains. Among these properties are (1) the degree of invasiveness in vaccinated persons, as shown by the development of viremia, (2) genetic stability in the course of multiplication in man; and (3) degree of spread from vaccinated to non-vaccinated contacts.

Two reports indicated that there was considerable difference in the degree of neurovirulence between the three sets of strains being studied, as demonstrated in comparative tests by both intracerebral and intraspinal inoculation of monkeys The differences were particularly apparent in the case of monkeys inoculated intracerebrally

Recent investigations indicate that the study of genetic "markers" related to virulence may prove very useful It was emphasized that more work should be done with these new markers.

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either a higher dosage of each virus type is used in a trivalent preparation or when the preparation is fed more than once, the results were at least as good as, or better than, those achieved by feeding the strains consecutively.

Dissemination or cross infection

The degree of spread of infection to contacts of those vaccinated varied considerably in different situations, but in families and in closed institutions, spread was common. In countries with a low degree of natural immunity in childhood, considerable spread may occur in the non vaccinated population. On the other hand it appears that in communities where immunity is acquired at an early age, the spread of the vaccine virus may be restricted. It has not been possible to follow clearly the natural spread of attenuated poliovirus after its first or second passage.

Safety

Since the development of these vaccines each has been widely used under a great variety of circumstances. Studies of great magnitude involving administration of vaccine on a community-wide basis to hundreds of thousands of persons have been reported from Africa, Asia, Europe and Latin America and two trials involving several millions in the USSR. Smaller and therefore more carefully controllable, studies have been reported from Europe and the United States. The degree of thoroughness with which orally-vaccinated persons and their contacts have been observed for evidence of adverse effects has varied greatly in different studies, however, no evidence has been produced that the use of any of the vaccines has been followed by either paralysis or ill defined illnesses in either group greater in number than has been observed in a control group or in the community at large among non-vaccinated persons.

Effectiveness

Although the studies have uniformly shown significant increases of antibody for all three types of virus, and therefore presumed protective value, few of the studies have been conducted under conditions which made it possible to shed light on the value of the vaccines in preventing paralysis. This has not been due to defects in the studies, but rather to the known low attack rate of paralytic poliomyelitis, the extreme variability in the occurrence of the disease, and the shortness of time that has elapsed since the vaccines have been fed. Therefore, in most of the studies there has been no attempt to assess effectiveness other than by measurement of increase in antibody. In a few instances, however, it has been claimed that use of the vaccines was followed by low rates of poliomyelitis, either in those vaccinated or in the community which were consistent with those which might have been expected as a sequel to use of an effective vaccine. Conversely, no data as to increased or continued high incidence of poliomyelitis have been reported which would indicate a lack of effectiveness. While there are certain differences of opinion as to interpretation, there is general agreement that more studies and longer periods of observation are required before definite conclusions as to the value of the vaccines in preventing paralysis can be reached on the basis of field observations. However, taking into account our present knowledge of the immunology and pathogenesis of poliomyelitis, it is generally agreed that assumption of effectiveness is reasonable providing virus excretion and antibody response has occurred.

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